The role of protein reserves in egg formation in birds

By

Richard George Selman, B.Sc.

A thesis submitted for the degree of

Doctor of Philosophy

Department of Zoology

University of Glasgow

December 1994

Declaration

I declare that the research described in this thesis has been carried out by myself unless otherwise cited or acknowledged. It has not, in whole or in part, been submitted for any other degree.

R.G. Selman

December 1994

Acknowledgements

Many thanks go firstly to David Houston, for supervising this project and helping to turn my raw notes into a finished thesis. James Currall, Neil Metcalfe, and Pat Monaghan provided valuable statistical advice and David Thomson kindly ran my chick growth data through the Genstat program. Richard Phillips and Scot Ramsay have both looked after the breeding birds and taken measurements while I have been away, and Caroline Askew assisted in the egg dissections. Margaret Hunter has helped with the typing and proof reading and has been wonderfully supportive during the final slog. I thank my parents for checking rough draughts and Dorothy, Rena, John and the staff of Animal Services for looking after the birds. I would also like to thank Graham Coombs and Felicity Huntingford for use of the departmental facilities. The staff were all very kind and helpful throughout. I thank particularly my fellow postgraduate students for advice and support through all the rigours of a Ph.D., but especially Isabel, Kate, Iain, Mustafa, David, Parviz, Liza, Nigel, Julie, Jayne, Fraz, Graham, Chris, Wylie, Mark, and Belen. This study was funded by an award from the Natural Environment Research Council (GT4/91/TLS/22).

Dedication

I dedicate this thesis to my parents, who instilled in me the value of an education and set me off on the biological trail, and to my grandfather, who after 97 years, lived just a day too short to see it completed.

Contents

Abstract

A technique was developed to estimate the muscle condition of live zebra finches by modelling the profile of the pectoral muscles over the sternum. The relationship between muscle condition, before and after laying, the timing of breeding, and the number and mass of eggs produced was examined. Females lost protein during egg production, but daily seed intake was reduced at this time. This was lower for large clutches than for small clutches. This suggests a reliance on body reserves for the nutrients required in laying a clutch of eggs. No aspect of egg production could be related simply to the amount of protein lost by females during egg formation, but the timing of breeding was related to body condition. This must be the result of variation in the fat reserves of females. The effects of both protein reserve size and quality were therefore compared during egg production. Birds with a reserve of 'high quality protein' laid heavier eggs and larger clutches than those with one of 'low quality protein'. There was a positive relationship between the loss of protein from the pectoral muscles and the clutch size produced by 'high quality protein' birds. These produced large clutches and large eggs. 'Low quality protein' birds all lost large amounts of protein, but laid only small clutches and small eggs. Dissection of the eggs revealed no differences in the relative sizes of egg components with varying egg mass, but shell dry mass was lower for 'low quality protein' birds than 'high'. Very small eggs, produced only by 'low quality reserve' females, did not develop. Amongst the other eggs the probability of hatching was related to egg mass. Hatching size was related to egg mass, but not hatchling condition. The correlation between egg mass and chick size remained through to fledging for chicks reared by birds with a 'high quality protein' reserve. Egg production was therefore greatly affected by the quality as well as the size of female protein reserves.

Chapter 1

General Introduction

The use of protein reserves during egg formation.

Female birds of a wide range of species have been shown to lose protein during breeding. Some studies have shown large losses during chick rearing, when time for the parents to feed themselves is short. This is the result of a lack of energy or proteins for the natural turnover of body maintenance. Significant quantities, however, may be lost during egg formation, as found in the red-billed quelea, *Quelea quelea* (Jones and Ward, 1976), *Camaroptera brevicaudata* (Fogden and Fogden, 1979), the house sparrow, *Passer domesticus* (Jones, 1991) and the lesser snow goose, *Chen caerulescens* (Ankney and MacInnes, 1978). The muscle condition during the egg laying period has been examined for 30 species of bird, and in 22 of them there was a significant loss in mass (Houston, Donnan, Jones, Hamilton and Osborne, in press). It is therefore possible that the size of the body protein reserve of a laying hen might limit reproductive success under certain conditions.

The protein for a clutch of eggs must come from the diet or from reserves within the body. The availability of protein for egg provisioning therefore depends upon the time available for foraging, the quality of the diet, the size and quality of any body reserves and a bird's ability to mobilise these rapidly. Wherever one of these factors is severely restricted there could be a resulting reduction in the number or mass of eggs produced and therefore the number or quality of young fledged.

There are a number of advantages to having a body protein reserve: there may be relatively little time available for foraging when nest building and territory defence

are important; the diet may provide severe seasonal restrictions to breeding because it must provide for the maintenance of health, the growth of the reproductive organs, and egg formation. A protein reserve could then increase seasonal reproductive success by allowing egg formation to occur prior to the peak of food availability, so that the growth and maturation of the new generation would occur during the most favourable time, or allowing an earlier start to the season for birds in a competitive environment. A protein reserve might also allow a bird to concentrate on foraging for energy rich foods, or allow the rapid provision of amino acids that are at low concentrations in the diet but high concentrations in the eggs and consequently act as a potentially limiting factor in egg production. Protein reserves therefore have the potential to affect egg production in a wide range of species.

The relative importance of diet and protein reserves for a laying hen.

Protein reserves are known to be used by a wide range of species during egg production, but the importance of this source of nutrients, in comparison with provision from the diet, may depend on the conditions in which a species breeds and variation in conditions between seasons for a single species. For instance, many polar nesting birds must produce and rear their young in a short season of food availability and hospitable conditions, bounded by the build-up of ice and storms. Species such as the lesser snow goose make a long migration to their breeding grounds and arrive when there is still snow cover. They feed very little during egg formation and therefore produce their eggs by drawing heavily on body reserves of both fat and protein (Ankney and MacInnes, 1978). A female eider duck (*Somateria* species) must endure a similar situation. She nests near the Arctic Circle and incubates her eggs without the help of her mate. If the eggs were left for long they might chill and lose their hatchability. The females therefore cease feeding whilst laying the eggs, normally resuming feeding only when incubation

has been completed. Body reserves appear to form an important supply of protein for the eggs (Korschgen, 1977).

The white-bellied swiftlet, *Collocalia esculenta* however, appears to get enough fat and protein for egg production from its insect diet. No loss of reserves was observed by Hails and Turner (1985) during laying. Either the birds cannot, for some reason, use reserves of fat or protein, or clutch size and egg size were, in this study, limited by other factors to a degree that precluded the need to draw on body reserves. In this case the diet appeared to provide all the necessary nutrients for egg production. A more in-depth biochemical study would determine whether small quantities of particular nutrients are sequestered for use in egg production even though a significant loss of protein reserves has not been found.

The aforementioned are extreme examples. Many studies have shown a decline in body protein during egg production (Houston, Donnan, Jones, Hamilton and Osborne, in press). Those that have not, excepting the swiftlet study of Hails and Turner, show a reliance on lipid stores that allow foraging for a diet high in protein, as found for many waterfowl (Ankney and Alisauskas, 1991). But do the protein demands of laying really limit reproduction? Ankney and MacInnes (1978) demonstrated that the lesser snow goose lays a clutch size corresponding to the size of her fat reserves on arrival at the breeding site. Body reserves are critical in this species for both egg formation and incubation. Some females died at the nest as a result of starvation near to the date of egg hatching and so adult survival may be affected by the levels of fat or protein. In contrast, Jones and Ward (1976, 1979) came to the conclusion that clutch size in the red-billed quelea was determined by the rate of use of the protein reserve, which depends on a female's protein intake. Also in poor years, colony desertion may occur as a result of the low status of fat and protein reserves: dead females have even been found at a nesting site during the laying period. Houston, Donnan and Jones (in press *a*) have calculated that

captive zebra finches (*Taeniopygia guttata* Vieillot, also known as *Poephila guttata*; see Clayton & Birkhead, 1989) given an excess of seed, produced 95% of their egg proteins from body reserves. This is remarkable for a bird given food *ad libitum*. This was not due to a digestive bottleneck caused by limited daylight hours for feeding, because experimentally increasing day length did not result in increased food intake. The use of reserves may depend greatly on the food available in a particular situation, and therefore may vary between sites and seasons. Body reserves could release a bird from the constraints of a restrictive diet, allowing breeding to occur when the diet would otherwise prevent it.

There is therefore strong evidence for the use of protein reserves during egg formation, but there is very little direct evidence for the limitation of egg formation by protein reserves or their use as a cue for breeding. This has been restricted to only a few species: the red-billed quelea (Jones and Ward, 1976); the lesser black-backed gull, *Larus fuscus* (Bolton *et al.*, 1993); and the American coot, *Fulica americana* (Alisauskas and Ankney, 1985). This study is intended to examine the use of protein reserves by the zebra finch because further studies are required to determine how protein reserves are used during egg formation by a wide range of species.

Reasons for a loss of protein during laying.

It has so far been demonstrated that many species do reduce body protein levels during egg production, but why does this occur? Firstly, the female may have to reduce the time she spends foraging for food when laying, and her food intake may then be inadequate to satisfy her energy demands at this time. If this were occurring, however, we would not expect body fat to double whilst protein is in rapid decline, as found in wild *Cameroptera* (Fogden and Fogden, 1979) at the onset of laying. Protein, therefore, is not mobilised as an energy source alone. A

second explanation is that a decrease in activity during the laying period could cause atrophy of the muscles, releasing proteins. Houston, Donnan and Jones (in press *a*) have shown a decrease in the activity of captive zebra finches during this period. In the same paper, however, they demonstrate that not only muscle proteins are lost, but also those of the liver and gut. Atrophy alone could not explain this protein loss. A third explanation for the loss in female muscle condition is that the protein is required for oviduct and follicle growth, and for the provision of nutrients to the developing eggs. There is some evidence for this.

Donnan (1994) found that the days of highest protein requirement for the reproductive organs of zebra finches correlate with those of greatest protein loss from the muscles. Moreover, only a small proportion of the protein requirements of a clutch was provided in the diet (Houston, Donnan and Jones, in press *a*). The link between body protein loss and egg formation was formalised when Houston, Donnan and Jones (in press *b*) showed that radioactive sulphur-labelled methionine taken in through the gut was deposited in the flight muscles. When the birds bred this was then used to form the developing eggs. Also, a sarcoplasm protein of high molecular weight, isolated from the flight muscles, showed a greater decline during egg formation than other body proteins. Electron micrographs of the pectoral muscles of female zebra finches after laying show denser packing of the muscle fibres than in pre-laying birds, indicating a loss of sarcoplasm (Houston pers. comm.). Kendall, Ward and Bacchus (1973) have also demonstrated a selective loss of sarcoplasm protein in the red-billed quelea, but in the house sparrow Jones (1991) found a significant loss of the myofibrillar fraction, not the sarcoplasm.

Kendall, Ward and Bacchus (1973) suggested that there are labile, non-contractile proteins in the sarcoplasm of some species that could form a reserve of amino acids to buffer against times of protein stress, such as moult, reproduction, migration or

starvation. During egg formation, where a large amount of protein is required in a very short time, this may be particularly useful.

Project development.

We can therefore put forward an hypothesis stating that birds which begin breeding with the highest protein condition may have the potential to lay the largest clutches, or the largest eggs, or to lay earlier than other birds. The standard method for comparing protein status is by post-mortem analysis. However, to test this hypothesis an estimate of the protein condition of live birds is required in order to then compare this with the subsequent reproductive performance. In chapter 2 the development of a method of estimating pectoral muscle volume in live birds is recorded, which correlates well with pectoral muscle lean, dry mass. The pectoral muscles were chosen because they have been implicated as a potential site for protein storage (Kendall, Ward & Bacchus, 1973) and they form the largest block of muscle on a bird.

Chapter 3 investigates the relationships between female muscle condition, seed intake, the number of eggs laid, their mass, and the timing of laying. The value of a reserve, however, may not vary in direct proportion to its size, but also its quality. Chapter 4 determines to what extent protein reserve quality can affect a female's laying success after accounting for differences in reserve size. Chapter 5 extends this approach to record whether the reserves of a laying female affect an egg's hatching success, as well as the growth and survival of the chicks produced. The effects of egg mass, egg quality, and incubation behaviour are also considered. Chapter 6 is a general discussion of the implications of the study.

This thesis does not attempt to determine whether protein reserves are the primary factor controlling clutch size in the field. In order to establish the importance of

any factor, one must first determine whether that factor has the potential to affect reproduction and to examine how it could act. This project aims to provide some of that basic information for the use of protein reserves in egg formation, in order that future field research can be undertaken with a greater knowledge of the background information. For this reason it was based in the laboratory where the conditions can be kept constant and breeding trials can be conducted all year round. In the field it is very difficult to target dietary supplements on particular individuals and to time this provision so that it does not take place during egg formation. In the zebra finch the follicles take 4 days to mature. The first outward indication of egg production is the laying of the first egg. It is therefore not possible to confine supplements to the pre-laying period in a wild population, unless feeding is confined to a period remote from the breeding season. Even this would create problems when studying an opportunistic breeder such as the zebra finch because the provision of extra food may allow the birds to breed during the time of supplementary feeding. Nilsson and Svenson (1993) made a field study of blue tits, *Parus caeruleus*, in which two groups of birds were fed supplements either after the start of laying, or both before and during laying. This showed an advance in the start of incubation behaviour in the first group, and earlier laying in the second. This is a useful technique in the field, but is far from ideal. The design cannot distinguish between a change in behaviour due to an increase in food supply, or due to a change in body condition. It therefore provides an ecological assessment, but not a mechanistic one. By using a captive population individual birds can be monitored daily under controlled conditions. A bird's full history is also known and its diet can be changed prior to egg formation because the sexes can be segregated until breeding is required. Small effects that might be masked by variation in a number of other variables may become more noticeable when the birds are kept in controlled conditions. This captive study allows the estimation of the size of body protein reserves and general body reserves of individual birds, both before and after laying, in order to determine their effects on egg formation.

The study species.

The species chosen for study in this project was the zebra finch. This Australian arid-land bird is considered to be an opportunistic breeder, reproducing whenever conditions allow. It therefore breeds well in captivity at any time of year when given favourable conditions of food, light and warmth. The natural diet is almost entirely granivorous, even during breeding (Morton & Davies, 1983; Zann & Straw, 1984), and therefore comparatively low in protein content. The use of the pectoral muscles as a reserve of protein has been demonstrated in this species by Houston, Donnan and Jones (in press *b*). We see, therefore, that it is an unusual, but ideal species with which to study the use of protein reserves under controlled conditions.

Colony Maintenance.

The temperature in the rooms where the birds were housed was maintained at 23 $\pm$ 2°C and the lighting regime was 12:12 hours light:darkness. All the birds used were of similar age and breeding experience to avoid any confounding effects on laying performance. Unless otherwise stated, they were one year old and had not previously bred. When not involved in experiments, the birds were kept in flight aviaries with the sexes segregated. The diet was Haithe's Foreign Finch Mix, a seed mixture, and Haithe's Conditioning Food, which has a higher protein content to keep them in good condition. The cages used for breeding were all identical, measuring 60 x 50 x 40 cm, arranged in pairs with a removable partition between. The diet differed among experiments, but was selected from the mixed seed and the conditioning food mentioned, chicken (*Gallus gallus*) eggs and *Panicum* millet. Food and water were replenished daily except during the seed intake experiment, in which seed was provided every 2 days. Cuttlefish bone and grit were provided in all cages and replenished when necessary. ICI Forest Bark was used as a litter on

the cage floors except during the seed intake experiment (chapter 3), when newspaper was used in order that any spillage of seed or chaff onto the floor could be recorded. The bark was replaced as necessary, but not during the experiments, to minimise disturbance at the nests.

For breeding, pairs of males and females were placed in adjacent cages separated by a partition, around which the birds could hear each others' calls. They were left for about a week and then the partition was removed and the pairs allowed to mix. The partition was then replaced so that a breeding pair occupied each cage. This method allows a certain amount of mate choice for at least one of the pairs, to ensure a reasonable breeding success. Cages were fitted with a nest box approximately 12.5cm square, its lid hinged to allow observations of the nest contents. Wood shavings were placed in the bottom of the box and nesting material, dried and fresh grass, were provided in the box and the cage.

All the birds were marked with individually numbered red plastic rings supplied by Hughes. Burley, Krantzberg & Radman (1982) have suggested that the behaviour of zebra finches may be affected by ring colour, so only a single colour was used.

Chapter 2 - A technique for estimating pectoral muscle protein condition from breast moulds of small passerine birds.

Introduction.

Studies of the utilisation of resources at particular stages of an avian life cycle require an assessment of bodily nutrient reserves as well as the quantities entering the body in the diet. These are usually based on a combination of body mass and size measurements. The mass of a bird at any given time will be determined partly by its overall skeletal size and partly by the size of its soft tissues, relative to its skeletal size. The mass of these soft tissues is likely to fluctuate in response to changes in food supply. A bird in 'poor' condition will have, for its given skeletal size, a lower mass of muscle and fat than a bird in 'good' condition. Sibly, Jones and Houston (1987) showed that external body measurements (dry mass corrected for body size) are useful for estimating the fat levels, but not the protein levels, of lesser black-backed gulls. Since changes in fat levels are often much greater, by mass, than those for protein, mass adjusted for body size may be useful for determining the effects of fat reserves in birds, or as an index of the general nutritional state of a bird. Fat and protein reserves may, however, serve different functions. In the first days of egg production the protein levels of female *Camaroptera* fall whilst fat reserves increase (Fogden and Fogden, 1979), therefore levels do not always rise and fall in tandem. For this reason they should be measured independently.

To determine fat or protein condition accurately post-mortem analysis is required. Whole body protein and fat levels can be determined by Soxhlet extraction of lipids from whole carcasses (Hails and Turner, 1985; Ward, 1969), or from aliquots following carcass homogenisation (Hohman and Taylor, 1986). In a number of studies, protein condition has been estimated by examination of the pectoral muscles alone (Houston, Jones and Sibly, 1983; Jones and Ward, 1976). These comprise about

half the total mass of the skeletal muscle in the body (Ward, 1969) and form a discrete muscle block which is easily dissected out. They have also been implicated as a major site for protein storage (Kendall, Ward & Bacchus, 1973). This technique does not, however, allow us to relate an individual's nutrient status to its subsequent behaviour, or allow us to monitor changes by repeated measurement. To do this requires a non-invasive method.

There are presently a number of methods to estimate protein condition in a live bird. Sears (1988) has successfully demonstrated the use of ultrasound to measure the thickness of the breast muscles of the mute swan, *Cygnus olor*. Breast muscle thickness was accurately related to the lean, dry mass of the muscle. Although a range of probe sizes is available, the technique cannot be used on a bird so small as a zebra finch because the method relies on the reflection of a sound wave off the sternum. The sternum of a zebra finch is too thin to reflect the sound waves, which go straight through the body (Houston, pers. comm.).

An alternative method uses the electrical conductivity of the body to determine lean body mass, since the electrical conductivity of lipids is about 4-5% that of lean tissues, body fluid and bone (Pethig, 1979). Body fat levels are determined by the subtraction of lean body mass from whole body mass. Walsberg (1988) tested this method on a range of birds and rodents with body weights of between 14.6 and 627g, but the data for lean body mass and the electromagnetically determined lean body mass index fit a second order polynomial equation both for rodents and for birds: the heavier an animal, the greater the difference in conductivity readings for a single unit of change in lean mass. Birds of less than 20g lean body mass all register similar conductivities (Castro *et. al.*, 1990). The technique is therefore not useful for work with zebra finches, which are mostly between 11 and 16g.

A good alternative is to adapt the method of Bolton, Monaghan and Houston (1991), developed for use with the lesser black-backed gull. They used solder wire which was bent over the keel bone and pectoral muscles to form a profile of the pectoral muscle shape. A bird in "good muscle condition" will have pectoral muscles which bulge out from the sternum and keel, whereas a bird in "poor muscle condition" will have muscles which fall away from the keel, leaving the bone protruding. There was a strong correlation between keel length and depth, and so measurements of keel length taken on a live bird were used to estimate keel depth. The combination of keel depth and keel profile provided an estimate of the cross-sectional area of the pectoral muscle block. An index of pectoral muscle volume was produced by combining this with the length of the sternum and coracoid, which is approximately the length of the pectoral muscles and which can also be measured on a live bird. The index correlated well with pectoral muscle lean, dry mass.

The solder wire method was found to be impractical to use on a bird so small as a zebra finch (Houston, pers. comm.) because only thin wire could be used, which did not accurately retain the muscle shape. The theory of the technique was, however, applicable to the development of a new method to profile the shape of the pectoral muscles of small birds. This chapter describes such a technique. A mould of the shape of a bird's breast was taken, from which an index of pectoral muscle volume was derived. This was related to pectoral muscle lean, dry mass on a small sample of carcasses. The equations derived could then be used to estimate the pectoral muscle lean, dry mass of live birds. The method here was tested on zebra finches, but it could be adapted to any small species of bird, given a sample of carcasses with which to set up the predictive equations.

Methods and results

Measurement of the pectoral muscle profile.

Each bird was first weighed, to 0.01g, on a Precisa balance. Impressions of the pectoral region were made in a quick-setting alginate gel, Cavex CA37 Superior Pink, available from suppliers of dental materials. The alginate was mixed with water to give a smooth, runny paste, with which a small plastic tray was filled to a depth of two cm. Two minutes after mixing, the bird was placed breast downwards in the gel; one hand was used to hold the head and wings clear of the gel and the other the legs and tail. After about thirty seconds the gel solidified and the bird was lifted clear of the mould. Alginate does not adhere to the feathers and the process causes minimal stress to the bird. Once removed, the alginate retains an exact replica of the shape of the pectoral muscle region. This was then cast permanently in plaster of Paris ($CaSO_4$).

Assessment of body size

Pectoral muscle mass varies with body size so any index of "protein condition" must take account of this. Univariate measures, however, may explain only a small proportion of the total variation in body size, whereas principal components analysis can produce a "body size" factor reflecting the variance in a number of body measurements (Freeman and Jackson, 1990; Rising and Somers, 1989). Here four measurements were chosen: skull width at its widest point; tarsus length; sternum length from the "V" of the furcula to the posterior end of the keel; and the length of the radius and ulna. These represent the four major parts of the skeleton. Furthermore, they can be used for live birds; they reflect solely skeletal size and therefore are unaffected by variations in growth or wear; and together they all provided high loadings (>0.72) in a principal components analysis of fourteen female

birds. Correlation analysis was used to test whether overall body size, sternum length, or sternum-coracoid length was best related to pectoral muscle lean, dry mass.

The multivariate measure of body size (the first factor given by a principal components analysis of four skeletal measurements) clearly gave the best correlation with pectoral muscle lean, dry mass (r_{14}=0.847, P<0.001). Sternum length and sternum-coracoid length gave weaker results (r_{14}=0.626, P=0.017 and r_{14}=0.712, P=0.004, respectively). The multivariate measure was therefore used in the formation of the condition indices.

Carcass analysis

Fourteen female carcasses were used to derive the equations which were later applied to live birds. The birds were first weighed and measured. Their pectoral muscles were then moulded in alginate gel. The right pectoralis major and supracoracoideus were dissected out and dried in an oven to constant mass at 60°C. Pectoral muscle volume may reflect variation in both the fat and the protein content of the muscles. It is therefore important to determine whether the lipids make up a significant proportion of the variation in muscle mass. Lipid was removed by Soxhlet extraction (Sawicka-Kapusta, 1975) using chloroform solvent. The muscles were then redried at 60°C to constant mass and reweighed to determine the right pectoral lean, dry mass. Pectoral muscle lean, dry mass was calculated as twice this value. Lipid removed by Soxhlet extraction comprised between 1.2% and 14.5% of the total pectoral muscle dry mass (mean = 8.1%, SD = 3.3%, n = 14). This low deviation shows that the samples all had very similar levels of fat.

The sternum and keel were cut dorso-ventrally at half the distance from the furcula to the posterior end of the sternum. In order to estimate keel depth from measurements that could be taken on a live bird I correlated keel depth with six other body

measures: sternum length, head and bill length, skull width, tarsus length, radius and ulna length, and body size (table 2.1). Sternum length provided the best correlation and would therefore be used to estimate keel depth in live birds. Regression analysis provided the following formula for predicting keel depth in live birds:

Keel depth = 0.230 x sternum length - 0.577.

The angle made by the keel on the breast bone of 14 birds was measured at the cut edge. The mean keel angle was 128° and the standard deviation was $\pm$2°. 128° was therefore used to create area 3 (figure 2.1) on the breast mould cross-sections.

Derivation of an estimate of pectoral muscle lean, dry mass from the breast moulds.

The plaster casts of pectoral muscle profile were cut dorso-ventrally by bandsaw at the mid-point between the furcula and the posterior end of the sternum. Each cut surface was then placed downwards on cardboard and the breast outlines were traced with a pencil.

Three measures of cross-sectional area were tested (figure 2.1). For areas 1 and 2 lines were drawn perpendicular to the keel at the estimated keel depth for each bird (estimated from sternum length, see table 2.1), and at a constant depth of 7.5mm, respectively. I chose 7.5mm arbitrarily to give a quick and simple method. For area 3 a closer approximation to actual flight muscle cross-sectional area was gained by drawing in the mean angle at which the keel joined the sternum at the estimated keel depth. A computer plotter (a BBC Master computer with a Cherry Digitiser and puck) was used to calculate these areas for each half of a mould. Every shape was measured five times and the average was taken. Both halves of the mould were used because these gave different values as a result of the bandsaw removing a piece of the mould that is the width of the saw blade. By taking the mean for the two halves the

cross-sectional area of the pectoral muscles was estimated at a point halfway along the sternum. These were then converted to muscle volume estimates in one of two ways. Firstly by multiplying the area by sternum length (the length of the major part of the flight muscles), and secondly by multiplying the area by sternum-coracoid length (the full length of the flight muscles). Using three alternative cross-sectional areas and two muscle length measurements, six indices were thus produced. These were then correlated with actual pectoral muscle lean, dry mass (twice the value obtained for the right pectoral muscle). All the estimates gave good correlations with pectoral muscle lean, dry mass (table 2.2), but the best estimate was area 1 (which includes estimated keel depth, but not keel angle) by sternum length (figure 2.2). The relationship is as follows.

Estimate = 0.00058 x area1 x sternum length + 0.14373

Calculation of a protein condition index.

The best estimate of flight muscle lean, dry mass was regressed against body size (from principal components analysis) to give the expected flight muscle lean, dry weights for birds of different sizes. Muscle condition for an individual was then calculated as the positive or negative deviation in estimated flight muscle lean, dry mass from that expected for a bird of a particular size i.e. from the residuals (figure 2.3). This estimates the amount of protein a bird has, relative to its body size, and enables comparisons to be made between birds of differing skeletal size.

Accuracy

Estimated pectoral muscle lean, dry mass correlated well with the actual values following dissection (figure 2.4). The mean absolute deviation of the estimated from the actual values was 0.06 (SE=0.01, n=14) compared with a range in muscle mass of

0.26 to 0.86. This difference can be expressed as 10.27% (SE=2.33, n=14) of the actual value. An estimate of muscle condition was derived from the regression of the estimated pectoral muscle lean, dry mass on body size, derived from figure 2.5. This was repeated using the actual pectoral muscle lean, dry masses, giving the actual muscle condition. Estimated and actual muscle condition were correlated (figure 2.5). The mean absolute deviation of estimated from actual muscle condition was 0.06 (SE=0.01, n=14) compared with a total range of -0.17 to +0.19. These statistics do not properly test the model since a model tested on the original sample will inevitably produce convincing results of its accuracy. An independent sample was therefore used to test the technique rigorously.

Assessment of the accuracy of the technique using an independant sample.

An independent sample of 15 female zebra finches was used to determine the accuracy of the technique. Females were sampled to test the model because they are the subjects of this study. They were chosen to show the full range of muscle condition in the colony. None of these birds had been used in deriving the equations of the estimation process. Pectoral muscle lean, dry mass was estimated from the live birds, using the breast moulding technique. The pectoral muscles were then removed and their true pectoral muscle lean, dry mass measured, following the extraction of the lipid as detailed earlier, allowing a comparison of estimated and true values.

Significant correlations were found between estimated pectoral muscle lean, dry mass and actual muscle mass (figure 2.6). The mean absolute deviation of the estimates from the actual values was 0.06g (SE=0.02, n=15) compared to a range in muscle mass of 0.51g to 0.88g. This is 8.37% (SE=2.21, n=15) of the actual value. For muscle condition, the mean absolute deviation of estimated from actual values was 0.06 (SE=0.02, n=15) compared with a range of condition from -0.12 to +0.11. However, the mean of actual condition (i.e. the mean absolute deviation of actual

condition from zero) was 0.05 (SE=0.01,n=15), so a guess of zero condition would have provided slightly more accurate results for condition in this particular sample, but estimated pectoral muscle condition correlated with actual pectoral muscle condition (figure 2.7) so this method may be useful for determining muscle condition as well as muscle mass.

A model based on a larger sample

Having developed a model for estimating pectoral muscle lean, dry mass, and tested it on an independent sample, a final model was produced using both samples of birds (total n=29). With this larger sample size the model produced should give a more accurate estimate than that already tested.

The new model was produced by the regression of pectoral muscle lean, dry mass on area 1 x sternum length, giving the following equation:

Estimate = 0.00051 x area 1 x sternum length + 0.19649.

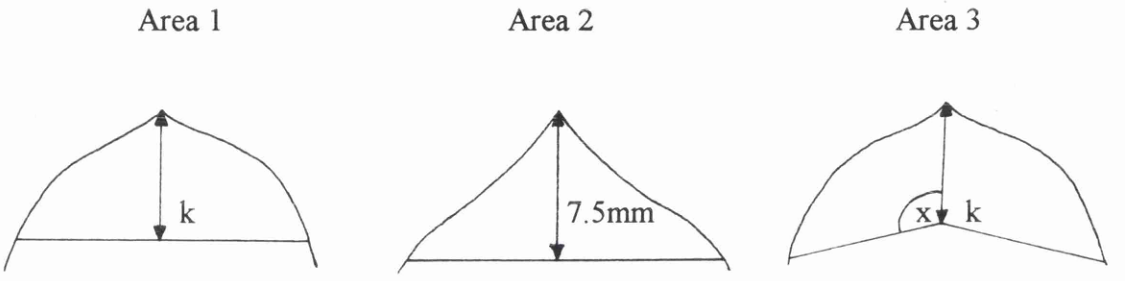
Table 2.1. Correlations of keel depth with six external body measures. The Pearson correlation coefficients (r) and their significances are given for a sample of 14 birds.

Variable	r	Significance
sternum	0.755	P=0.002
head and bill	0.689	P=0.006
skull width	0.644	P=0.013
body size	0.571	P=0.033
tarsus	0.251	P=0.386
radius and ulna	0.203	P=0.487

Table 2.2. The correlations of six estimates of muscle volume with pectoral muscle lean, dry mass for a sample of 14 birds. Pearson correlation coefficients (r) and significances are given.

Variable	r	Significance
area 1 x sternum	0.924	P<0.0001
area 2 x sternum	0.921	P<0.0001
area 1 x sternum-coracoid	0.917	P<0.0001
area 2 x sternum-coracoid	0.916	P<0.0001
area 3 x sternum	0.883	P<0.0001
area 3 x sternum-coracoid	0.880	P<0.0001

Figure 2.1. Three measures of pectoral muscle cross-sectional area. Areas 1 and 2 show the difference between birds in good (area 1) and poor (area 2) muscle condition.



k = estimated keel depth, x = mean keel angle.

Figure 2.2. Regression of actual pectoral muscle lean, dry mass on the best estimate of muscle volume (area 1 x sternum length). Regression coefficient r=0.924, n=14, P<0.0001.

$y = 0.14373 + 0.00058x$

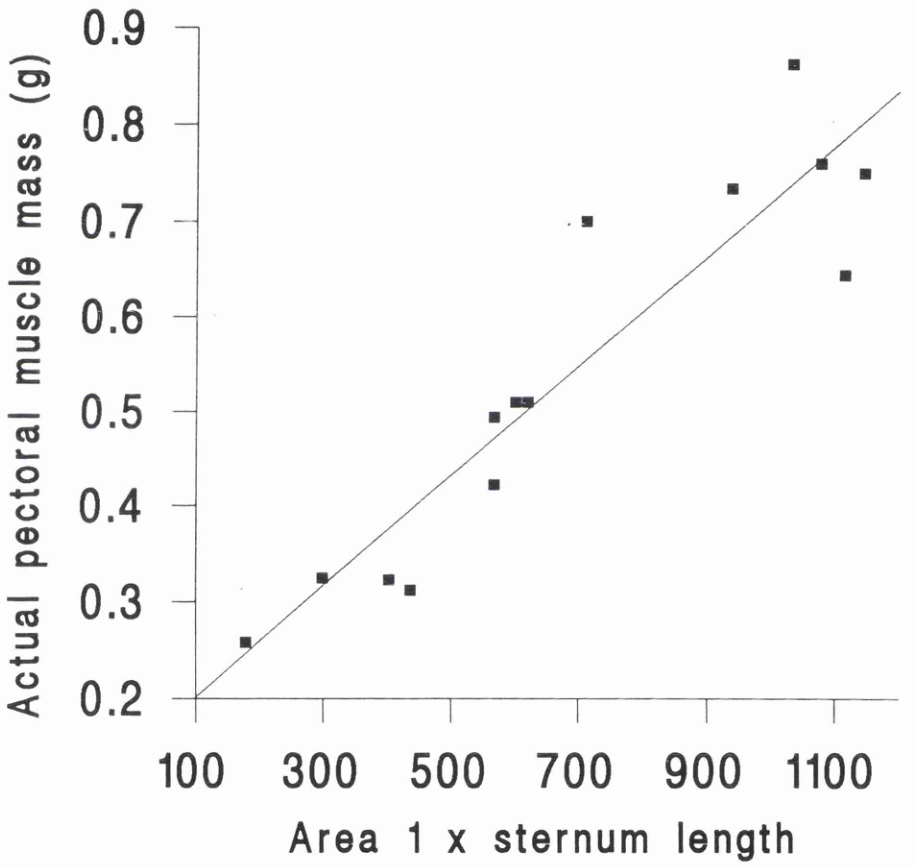


Figure 2.3. Regression of estimated pectoral muscle lean, dry mass on "body size". This was used to give an index of protein condition.

$y = 0.54529 + 0.15218x$

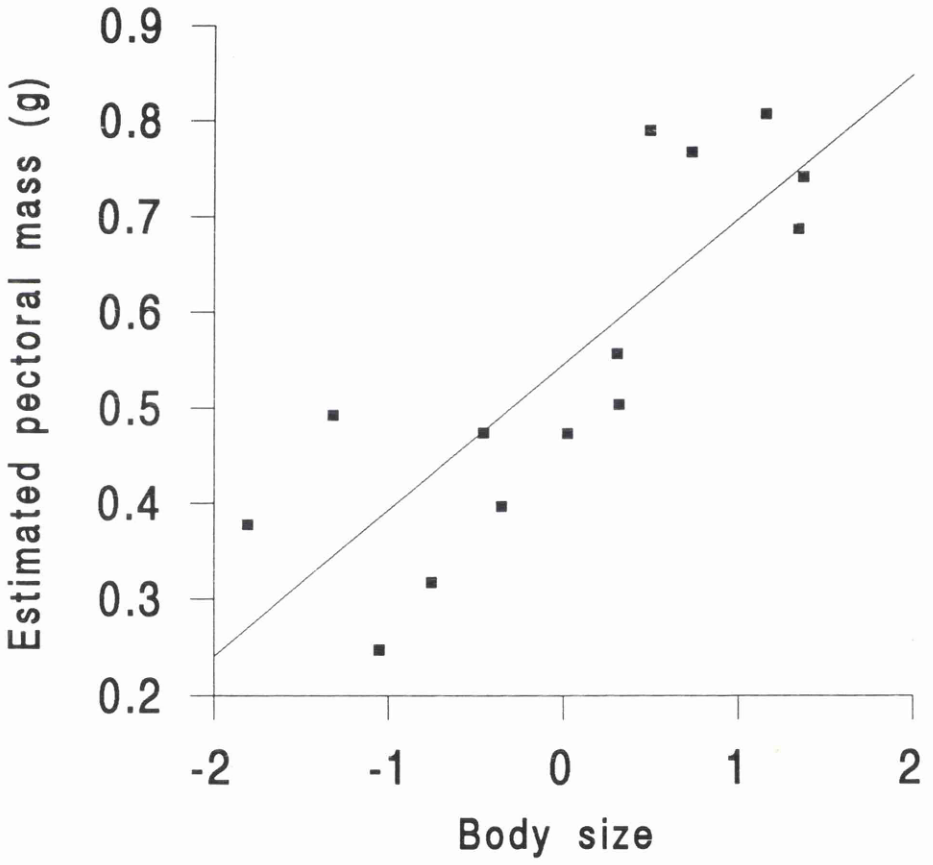


Figure 2.4. Correlation between actual and estimated pectoral muscle lean, dry mass for the sample used to set up the technique. The diagonal (estimate = true value) is shown. Pearson's r=0.924, n=14, P<0.001.

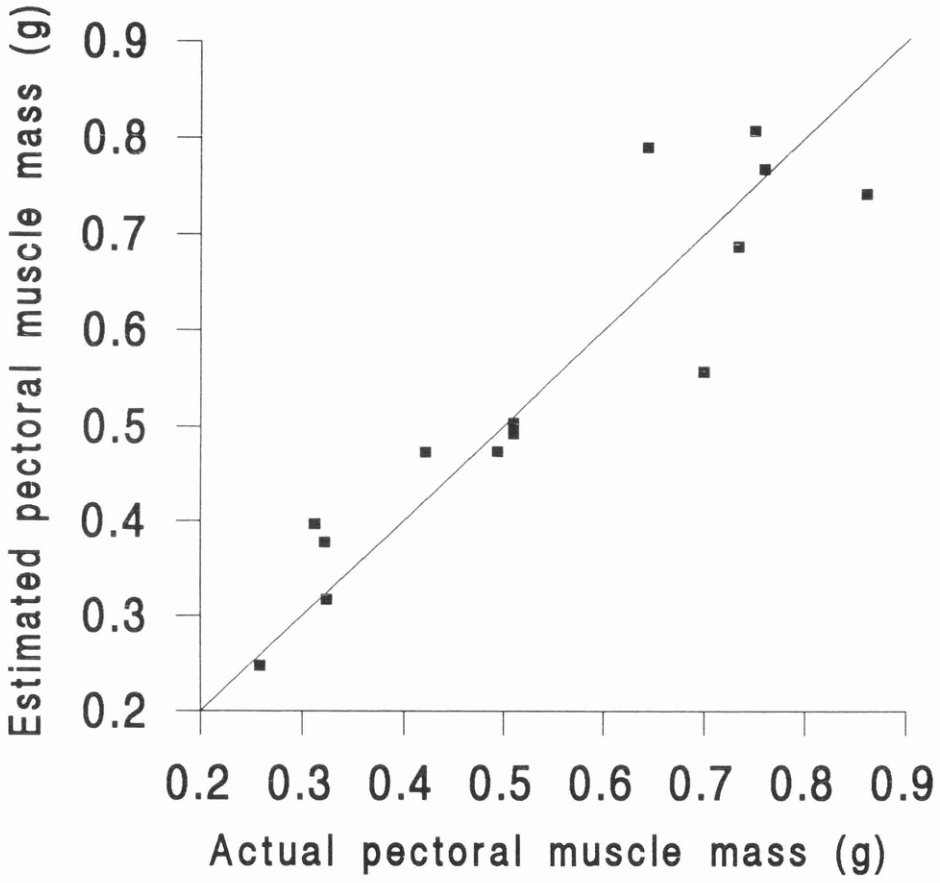


Figure 2.5. Correlation of actual and estimated muscle condition. The diagonal (estimate = actual value) is shown. Pearson's r=0.750, n=14, P=0.002.

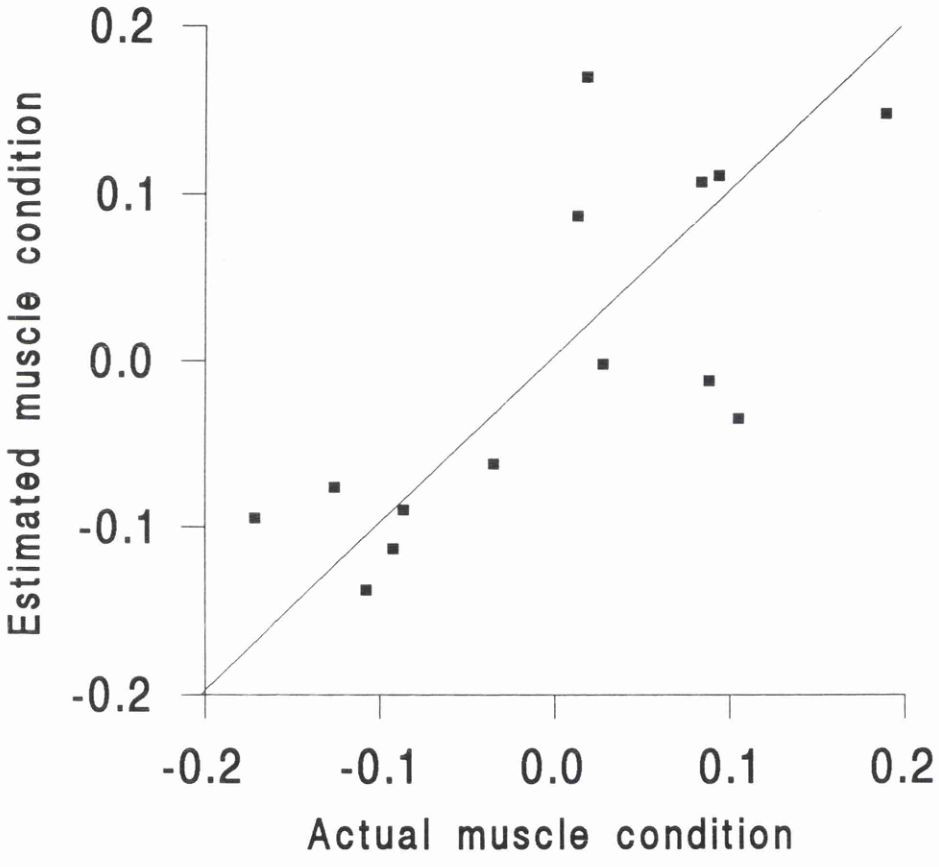


Figure 2.6. Correlation between actual and estimated pectoral muscle lean, dry mass for an independent sample. The diagonal (estimate = true value) is shown. Pearson's r=0.750, P=0.001, n=15.

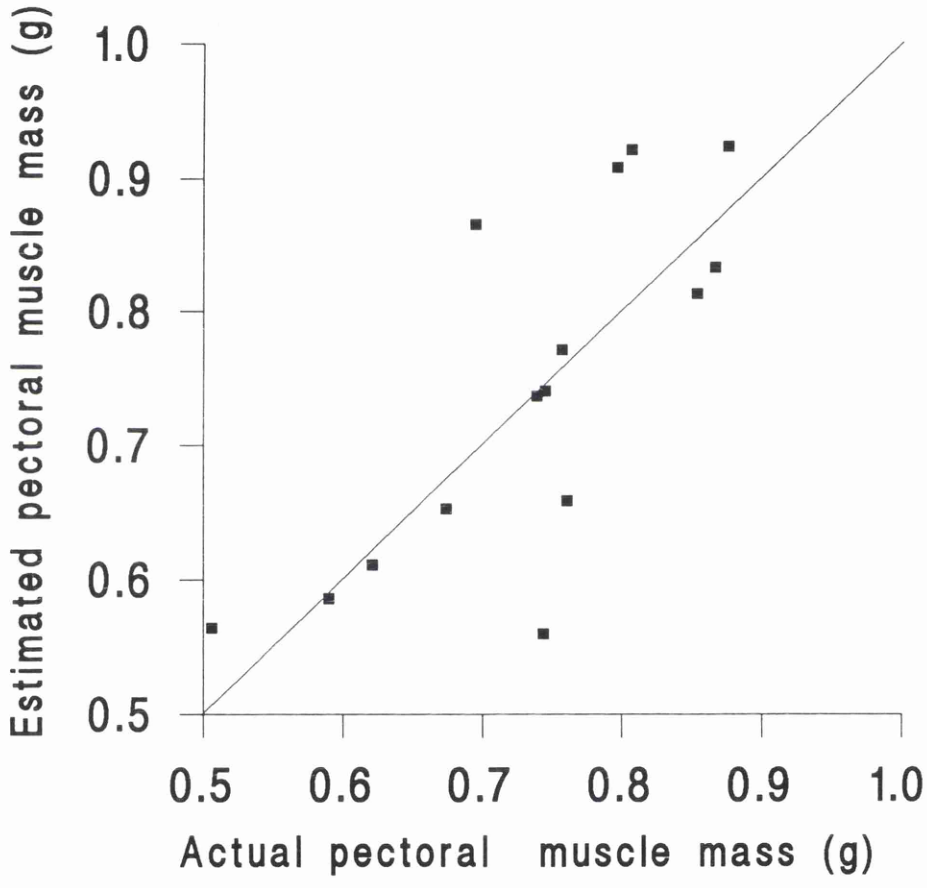
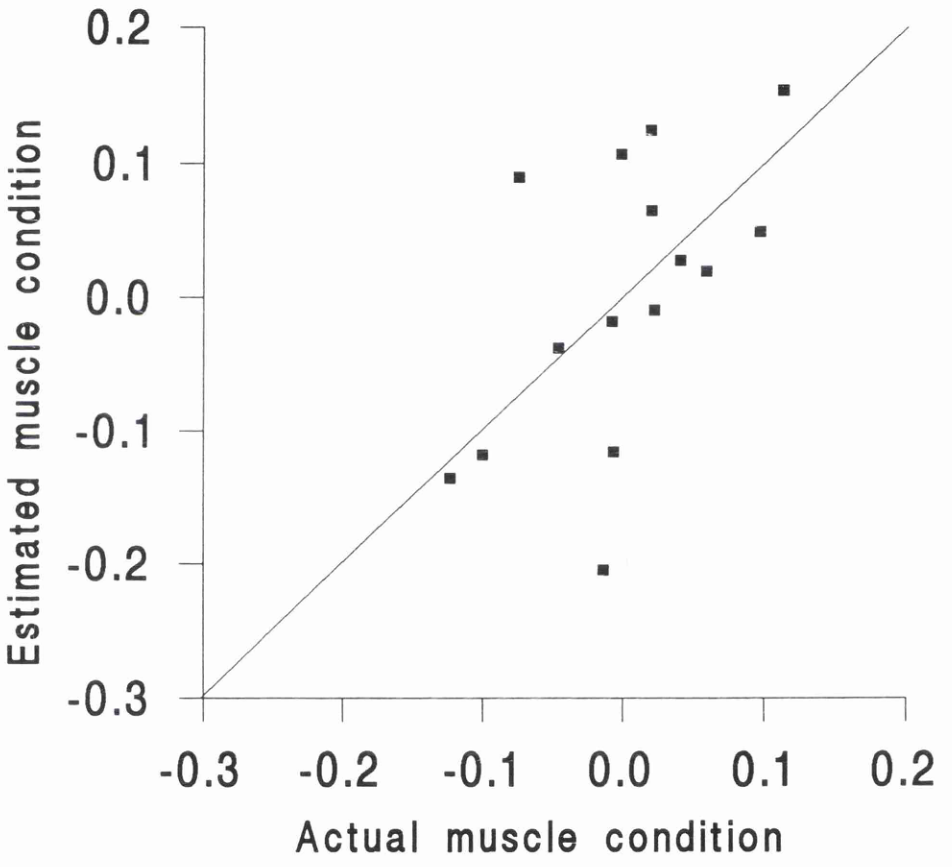


Figure 2.7. Correlation of actual and estimated muscle condition for an independent sample. The diagonal (estimate = actual value) is shown. Pearson's r=0.564, P=0.029, n=15.



Discussion

The technique presented here allows the determination of muscle mass in small birds, such as the zebra finch, for which no other method is available for use on live birds. The handling time for each bird is quite short, and when paired with a male, immediately after breast-moulding, the birds went on to breed, indicating that stress levels in the female are not unduly high. For larger birds the breast-moulding method would be better replaced by the use of solder wire (Bolton *et al*, 1991) since it is impractical to hold large birds in buckets of alginate. The use of ultrasound (Sears, 1988) or electrical conductivity (Walsberg, 1988) are also more appropriate for birds of greater than 20g. A major difference between the estimate of pectoral muscle mass used by Bolton *et al.* (1991) and that used here, is that Bolton *et al.* included fresh mass in the estimate. I have found that this would increase the correlation between estimated and actual values slightly, but significantly, but here it has not been included. Its use may appear to increase the accuracy due to a correlation between muscle mass and body mass in the sample. Fat and protein condition may be correlated for much of the time, but during egg formation they may not necessarily rise and fall in tandem. Fogden and Fogden (1979) and Jones and Ward (1976) have shown independent changes in fat and protein levels for the *Camaroptera* and red-billed quelea respectively. It is therefore wise to assume that in birds body fat and protein levels may not be correlated so an index of either fat or protein should attempt to exclude the effects of the other. The estimate of muscle mass used here is not affected by changes in the major fat deposits, which are not found at the point of muscle measurement, but would be affected by differences in the concentrations of intra-muscular lipid. However, Houston, Donnan and Jones (in press *a*) report that significant amounts of lipid are lost from the major fat deposits during the laying period, but not from the muscles of the zebra finch.

In this thesis, the levels of muscle condition may be compared with those of body condition to reveal differences in the use of protein and fat reserves, since muscle condition reflects only the level of protein, whereas body condition reflects the levels of both fat and protein. Any discrepancy between changes in the two indices would indicate that fat and protein levels are not changing in tandem.

Pectoral muscle lean, dry mass, estimated by the moulding technique, correlated highly with the values determined by dissection. Houston, Donnan and Jones (in press *a*) have found from dissections of captive zebra finches that on average 14% of female pectoral muscle lean, dry mass is lost during egg formation. For the breast-moulding method the mean difference between the actual and estimated pectoral muscle lean, dry mass was 8% of the actual values. The technique is therefore unlikely to be accurate enough to monitor muscle mass on a daily basis, but should prove to be a useful tool for comparing groups of birds or for determining the change in mass of birds over longer periods, such as from the beginning to the end of the laying or moulting period.

Some error was expected, due to differences in plumage thickness and the air trapped in the feathers, but the profiling technique nevertheless remains effective in estimating pectoral muscle lean, dry mass. The birds in this study were kept calm before profiling and all were handled in the same manner, so as not to affect the muscle volume as a result of muscle expansion during exercise. This may be important in ensuring the accuracy of the technique. Moult is continuous in zebra finches. This could affect profile measurement between individuals, but there is no moulting period to take into account. There may also be error due to variations in fat levels. Here I found very little subcutaneous fat overlying the pectoral muscles. It was deposited mostly in the omentum and the tracheal pit, where it will not affect the estimate of muscle mass. The plaster cast of muscle profile was measured half way along the keel bone, at a point which is unaffected by these two fat bodies. Variations in the concentrations of

fat within the muscles and the amount of exercise a bird takes might affect muscle density, and therefore the relationship between muscle mass and profile index. Such effects were minimised by keeping all of the birds in identical housing conditions for all of the experiments described in this thesis. Any effects due to differences in dietary treatments, or changes in behaviour through the laying cycle, can only be considered by testing their effects on muscle composition. This would require the sacrifice of a large number of birds, a sample for every change in protocol. Birds were only killed to set up the estimation model for muscle mass and condition. I did not consider further sacrifices to be justifiable. Fat within the muscle made up, on average, 8.1% of pectoral muscle dry mass. The standard deviation was 3.3% so the concentration of fat was similar in all of the birds. It is therefore unlikely that fat will significantly affect estimates of pectoral muscle lean, dry mass.

When comparing muscle condition between birds, care should be taken to profile them at similar times of the day: significant losses of pectoral muscle have been shown overnight for white-crowned sparrows, *Zonotrichia leucophrys gambelii* (Murphy and King, 1990) and for house sparrows (Jones, 1980).

Muscle condition was calculated as the pectoral muscle mass of a bird relative to its body size. When the estimate of muscle condition was compared with the "actual muscle condition", derived from the dissections, a lower correlation coefficient was given than for the comparison of estimated and actual pectoral muscle mass. This was because the differences between estimated and actual muscle condition were larger, in comparison with the full range of condition shown, than the differences between estimated and actual pectoral muscle lean, dry mass, relative to the range shown here, i.e. the effects of differences in mass are amplified when body size is accounted for.

Also, one should note that in the independent sample used to test the method the mean absolute deviation of estimated from actual muscle condition (0.06) was slightly

greater than that for a guess of zero (0.05). This could have occurred because the birds in the particular sample tested all fell within a narrow range of condition. The errors of estimation thus appeared great compared to the range of condition in the sample. It is therefore very important to estimate pectoral muscle mass with great accuracy to get a reliable index of condition because a small error in the estimation of muscle mass will have a great effect on the value of muscle condition obtained. The correlation of actual and estimated condition shows that this method of determining muscle condition may still be of some use. Further testing with an independent sample containing a much wider range of muscle condition would be wise.

Chapter 3 - The effect of protein reserve size on laying.

Introduction

Many studies have shown a loss of body protein reserves in female birds during egg production (reviewed by Houston, Donnan, Jones, Hamilton and Osborne, in press). Post-mortem studies have linked nutritional state with some aspects of reproductive state (Alisauskas and Ankney, 1985; Ankney and MacInnes, 1978). But, although clutch size can be determined from post-mortem examination, other factors such as egg mass and laying date cannot. In order to relate all these elements of egg production to the levels of body reserves it is necessary to use some measure of nutrient reserve in the live bird. An analysis of changes in body mass can indicate the changing state of body condition (Hornfeldt and Eklund, 1990), but fat and protein reserves do not always rise and fall in tandem (Fogden and Fogden, 1979; Jones and Ward, 1976) and should ideally be estimated separately.

The development of methods which overcome these problems (chapter 2, introduction) has brought new opportunities to study the changes in body fat and protein stores during times of nutritional stress, such as moult and breeding. Very little work has been done to determine the effects of protein reserves on egg production. This has been attempted by Bolton with the lesser black-backed gull (Bolton *et al*, 1993). Female pectoral muscle protein condition before laying correlated with clutch size, but not egg size. Protein condition may, therefore, affect egg production in situations of dietary stress. In this chapter I used a modification of the breast moulding technique of Bolton *et al.* (1991) (chapter two) to elucidate the relationship between protein condition and the initiation and cessation of laying in the zebra finch.

In this strict granivore, Houston, Donnan and Jones (in press *a*) have demonstrated that 95% of the protein requirements for a clutch may be obtained from body reserves. Seed intake did not increase at all during egg production even though abundant seed was available. Protein reserves are therefore very important in this species.

Here I confront two major questions in the control of breeding: what determines if and when laying begins? and what determines the reproductive output in terms of clutch size and egg mass? In a post-mortem study of red-billed quelea, Jones and Ward (1976) found that female protein reserves were higher just before breeding than at any other time during the year, falling rapidly during the first days of laying, and recovering during incubation. They proposed that an individual's protein reserves could provide proximate control to breeding. Four models were given (figure 3.1).

In scheme (a) all birds start to lay on reaching a threshold level of protein reserves. These are then depleted at a constant rate. The level of reserves at the close of laying reflects the clutch size laid. In (b) birds begin laying in a range of condition and deplete their reserves at a constant rate, but they all finish laying at the same minimum reserve level. Clutch size, again, is proportional to the amount of protein reserve used.

In both (c) and (d) all birds start to lay on reaching a threshold level of protein reserves and complete laying on reaching a minimal level of protein, or on approaching this level too quickly. Differences in clutch size therefore arise from variations in the rate of use of protein reserves. A bird with a high dietary protein intake may be able to use internal protein reserves at a minimal rate and therefore continue to lay eggs for a long period, resulting in a large clutch size. Schemes (c) and (d) differ in the shape of their decline curves. In (c) this is constant, the rate depending on the clutch size to be produced and the rate of dietary intake. Clutch size is determined early in the period of yolk formation. In (d) a decision is delayed as late as

possible, allowing a bird to adjust clutch size to suit any sudden change in food abundance. The rate of reserve use is kept to a minimum until clutch size is determined. For the red-billed quelea, Jones and Ward favoured schemes (c) and (d).

In this chapter, the use of thresholds in the decision to begin or to finish laying is investigated in the zebra finch. The first aim was to determine whether the decision to breed was affected by the size of the protein reserve. The second was to determine whether the number or weight of eggs laid was affected by protein condition before laying or after laying; or by the overall quantity of protein lost. The rate of protein loss could not be determined because the breast moulding technique is unlikely to be accurate enough to detect daily changes. The third aim was to compare the relative importance of female protein condition and food intake on egg production.

Methods

The effect of pre-laying protein condition on reproductive performance.

In this chapter I present the results of three breeding trials. The first compared female muscle condition when the birds were paired (obtained from the pectoral muscle profile) with reproductive performance (whether she bred or not, the timing of laying, clutch size, mean egg mass, and clutch mass). Clutch size is here defined as the number of eggs in a sequence with not more than two consecutive days without laying.

Seventy-three females and an equal number of males, all of either one or two years of age, were maintained on a diet of mixed seed and conditioning food for 2 weeks prior to pairing to bring them into good breeding condition. During this time the sexes were segregated, two males occupying one side of a double breeding cage and two females, the other. A hardboard partition divided the two sides. The birds were then paired and the females breast moulded to estimate pectoral muscle lean, dry weight, from which the index of muscle condition is derived (see chapter one). Four body measures were taken: skull width, tarsus length, sternum length, and the length of the radius and ulna. After pairing a diet of mixed seed was provided.

The relative importance of food intake and female protein condition on egg production.

The results of the first trial were disappointing because few correlations were found. I therefore made two changes in the next trial. The lack of any effect of protein condition on egg production could have been due to the similarity of the protein condition of the birds, all of which had comparatively large muscle volumes. To overcome this, females in a wider range of condition than usually found in the captive

colony were chosen. Seed intake was also monitored. A diet of only one seed type, *Panicum* millet, with a protein content of 12.9% (Houston, Donnan and Jones, in press *a*), was provided after the birds had been paired so that seed intake could be measured by the weight of food eaten in a given time period. A mixed seed diet would have allowed variation in seed choice between individuals, making it more difficult to determine protein intake because protein content varies among seed types. It was hoped that the relative importance of protein intake and the use of body protein reserves during egg production could be considered.

Fifty-four one-year-old females in a wide range of muscle condition were chosen from the colony for breeding. For two weeks the birds were maintained either on a high protein diet (mixed seed and conditioning food), a low protein *Panicum* diet, or a restricted diet of 2g of *Panicum* millet per day in order to reduce further their reserves [the average intake is 2.85g/day (Houston, Donnan and Jones, in press *a*)]. The weights of the restricted diet birds were monitored carefully to prevent them from falling to a dangerous level. Body weight was not allowed to fall below 85% of the mean mass of females considered to be in 'good condition' (mean=14.94g, SE=0.23g, n=69). This mean mass was measured from females in the colony prior to the experiment, when they were all in good body condition. Muscle condition was estimated for each female on the day that pairing took place and four skeletal measurements were taken as above. This provided a measure of pre-laying protein condition. The method is described in chapter 1.

After pairing, a diet of *Panicum* millet was provided. The seed intake of each pair of birds was determined in the following way. The seed bowls were changed every two days. The seed provided was weighed before introduction. The cages were lined with fresh newspaper at each change of food in order to catch any seed and chaff spilt by feeding birds in the proceeding two days. The old newspaper cage lining was carefully removed and the spilt seed, chaff, grit and faeces collected. The seed and chaff were

separated from the grit and faeces by first passing the litter through a sieve to remove large faeces, then by picking out grit and faecal deposits by hand from the remainder. The food intake for a two day period was calculated as the initial mass of seed in a bowl minus the combined mass of the seed remaining in the food bowl after two days and the mass of spilt seed and chaff collected in the newspaper cage lining.

The nest boxes were checked daily for eggs. These were weighed on the day on which they were laid. A Precisa 4 point balance with an enclosed weighing surface was used. Muscle condition was again estimated following laying, on the second day on which no egg was laid. To do so on the first non-laying day would have meant disturbing some females mid-clutch where a laying gap of a day had occurred.

No effects of the size of protein reserves on egg production were recorded in the second trial, with birds in a wide range of condition. The poor correlations could have been caused by the delay between recording muscle condition (at pairing) and the start of laying, during which time the muscle condition may have changed. In a third trial I therefore tried to record muscle condition nearer to the time of laying. Muscle condition was estimated every four days from the day of pairing until laying had begun. In this way it was hoped that a more accurate measure of muscle condition immediately prior to the start of egg formation would be obtained. The nests were checked daily and eggs recorded as for the previous trials. The post-laying muscle condition of the females was again estimated on the second day on which no egg was laid and the seed intake of each pair was recorded during egg formation.

Results

I used four measurements of body reserves. For clarity I have termed these as follows. **Body mass** is the fresh body mass, and **body condition** is the residual mass from the regression of mass on the body size index (figure 3.2a). Estimated pectoral muscle lean, dry mass is abbreviated to **muscle mass**, and the residual muscle mass from the regression of muscle mass on the body size index is termed **muscle condition** (figure 3.2b).

Results are presented here for three separate breeding trials which, as already outlined, differed slightly in diet and the time at which muscle mass was estimated. To summarise, the first trial used mixed seed, the other two *Panicum* millet. Muscle mass was estimated at pairing in trial 1, at pairing and again after laying in trial 2, and every four days from pairing to laying, and again after laying, in trial 3. However, in trial 3 no significant change in muscle cross-sectional area was observed for females breast-moulded at pairing (mean=56.13mm^2, SE=3.18) and just prior to the initiation of egg formation (mean=53.36mm^2, SE=2.58; t_6=1.35, 2-tailed P=0.226, paired samples t-test), nor in mass (mean at pairing=16.24g, SE=0.69; mean prior to egg formation=16.44g, SE=0.68; t_6=0.91, P=0.399). This shows that there was no change in muscle mass or body mass in the few days between pairing and laying. I have therefore only presented data on muscle condition at the time of pairing, because these are available for a much larger sample of birds. Post-laying results are available for the two *Panicum* trials only, and therefore have smaller sample sizes than those for pre-laying protein condition.

Twelve birds laid eggs less than four days after pairing. These females must have already begun rapid yolk deposition when they were paired. They may therefore have lost some reserves for egg formation before the "pre-laying" measurement. Also their

body masses would have included an enlarged reproductive tract, adding noise to the data. They were therefore removed completely from the analysis.

The results of the three trials have been pooled where the dietary treatment was shown by analysis of covariance to have no significant effect on the results. Differences between dietary treatments were found for clutch size, (mixed seed median=4 eggs, IQR=3, n=49; *Panicum* median=3 eggs, IQR=2, n=66; Mann-Whitney U test U=1234.0, Z=-2.210, P=0.027), and clutch mass (mixed seed mean=3.63g, SE=0.30, n=47; *Panicum* mean=2.82g, SE=0.20, n=52; t-test t_{81}=2.24, P=0.028, but not for mean egg mass (mixed seed mean=0.994g, SE=0.03, n=47; *Panicum* mean=0.978g, SE=0.02, n=52; t-test t_{97}=0.54, P=0.591).

Are body reserves associated with whether birds will breed or not?

There was no consistent evidence for a correlation between the levels of body reserves when the birds were paired and the subsequent decision to breed or not. However, there were some indications that a weak association might exist. On the mixed seed diet females that went on to breed had significantly greater body weights at the time of pairing than birds that did not breed (table 3.1). When differences in body size were included, to produce an estimate of pre-laying body condition, laying birds were in significantly better body condition than non-layers. However, on a diet of *Panicum* millet, no significant differences were found. In none of the trials were there any significant differences in muscle mass or muscle condition between layers and non-layers at the time of pairing.

Are body reserves correlated with the time taken for birds to start breeding after pairing?

The number of days between pairing and laying was related to female body mass at the time of pairing (figure 3.3). Body condition (body mass allowing for body size) gave a stronger relationship with the delay in laying (figure 3.4). These relationships may not be linear, but curved, or possibly linear, with a change of gradient at a threshold of about 16g body mass and 1.7 body condition score. The relationship is, however, significant. A better fitting regression would serve to increase the level of significance. Birds heavier than 16g, or with a body condition score of more than 1.7, almost all laid very soon after pairing. Lighter birds delayed laying for a period in negative proportion to their masses. When birds of more than 16g were removed from the analysis, the correlation between body mass and the delay in laying was not significant (Spearman's r_{91}=-0.135, P=0.202), but after the removal of birds with a body condition score of more than 1.7, the correlation between body condition and the delay in laying remained significant (Spearman's r_{89}=-0.258, P=0.015). The number of days between pairing and laying did not correlate with muscle mass (r_{108}=-0.010, P=0.920, figure 3.5) or muscle condition (r_{108}=-0.015, P=0.874, figure 3.6).

Are body reserves correlated with the numbers and masses of the eggs produced?

a) *Clutch size*

The body mass and body condition of the females at the time of pairing correlated strongly with the number of eggs subsequently laid on a diet of mixed seed (see summary table 3.2 and figures 3.7 and 3.8). On the *Panicum* diet no such relationship was observed, but there was a negative correlation between clutch size and the mass and body condition of females at the completion of laying (figures 3.9 and 3.10). No effects of pre-laying or post-laying muscle mass or condition were shown.

On both the mixed seed and the *Panicum* diet there was a negative relationship between clutch size and the number of days between pairing and laying (figure 3.11). Large clutches were laid soon after pairing, but small clutches were sometimes delayed and sometimes not.

b) Mean egg mass

Table 3.3 shows a similar pattern of results for the relationship between body condition and mean egg mass as found for clutch size. On the mixed seed diet mean egg mass was related to pre-laying body mass (figure 3.12) and body condition (figure 3.13). On the *Panicum* diet these relationships were not shown, but mean egg mass correlated with post-laying body mass (figure 3.14), and body condition (figure 3.15). Mean egg mass was not significantly related with the change in body mass or muscle mass over the laying period, or the loss of body mass per gram of egg laid (r_{44}=-0.253, P=0.098) or muscle mass lost per gram of egg laid (r_{44}=-0.111, P=0.475). There were no significant relationships between mean egg mass and muscle mass or muscle condition before or after laying.

c) *Clutch mass*

In the mixed seed trial, clutch mass was correlated with pre-laying body mass (figure 3.16) and body condition (figure 3.17) (table 3.4). In the *Panicum* trial this did not occur, but there was a correlation with post-laying body mass (figure 3.18) and body condition (figure 3.19). Clutch mass did not correlate with muscle weight or muscle condition, either before or after laying, or the change in muscle mass over the laying period. This mirrors the results for both clutch size and mean egg mass.

Food intake and egg production.

Breeding pairs ate less seed each day than non-breeders. The mean rate of intake for non-breeding pairs was 8.20g *Panicum* per day (SE=0.24, n=8), whereas breeders ate an average of 6.98g/day (SE=0.13, n=63) during egg formation. This is a significant reduction of intake by breeding pairs (t_{69}=3.23, P=0.002). Seed intake correlated negatively with clutch size (figure 3.20) and weakly with mean egg mass (figure 3.21), but was not related to the number of days between pairing and laying (Spearman's r_{63}=0.231, P=0.069).

There was a relationship between the rate of food intake and the change in body mass during the laying period (figure 3.22): large masses were lost by females in pairs which ate little. This was reflected in the effect of the rate of seed intake on post-laying female body mass (figure 3.23) and body condition (figure 3.24), but there were no relationships with the pre-laying values. The rate of seed intake during laying did not correlate with either muscle mass or muscle condition.

The loss of female muscle mass during laying was not associated with the output of eggs or the rate of food intake during laying, but was related separately to both the starting and the finishing condition of a laying bird (figures 3.25 and 3.26). Those with the most protein at pairing, lost the most during laying and those that lost the most protein, finished laying in the poorest condition. However, there was not a negative relationship between pre-laying and post-laying muscle condition, but a positive one (figure 3.27). The amount of muscle lost therefore does not simply relate to the muscle condition of a bird when it begins to lay or to the level at which it stops. The birds which lost the most protein both started laying in high condition and completed it in very low condition.

Table 3.1 - A comparison of the mean body masses and muscle masses of layers (L) and non-layers (NL), both before and after allowing for differences in body size. Independent samples t-tests show whether there were significant differences between the means for layers and non-layers. Significant correlations are shown in bold type.

	Mixed seed diet	*Panicum* diet
Pre-lay body mass (g)	**L=15.27, NL=13.84**	L=14.40, NL=14.83
	t_{60}=-2.41, P=0.019	t_{92}=1.21, P=0.229
Pre-lay body condition	**L=0.887, NL=-0.209**	L=-0.486, NL=-0.108
	t_{54}=-2.18, P=0.034	t_{92}=1.07, P=0.289
Pre-lay muscle mass (g)	L=0.72, NL=0.68	L=0.75, NL=0.79
	t_{54}=-1.24, P=0.222	t_{92}=1.56, P=0.123
Pre-lay muscle condition	L=-0.009, NL=-0.025	L=-0.002, NL=0.031
	t_{54}=-0.48, P=0.631	t_{92}=1.48, P=0.141

Table 3.2 - Spearman's correlation coefficients for the relationships between clutch size and the levels of, or changes in body reserves. Condition indices allow for differences in body size. A positive change in mass indicates an increase in mass. Significant correlations are shown in bold type.

Diet	Mixed seed	*Panicum*
Pre-lay body mass	**r_{48}=0.442, P=0.002**	r_{66}=0.113, P=0.366
Pre-lay body condition	**r_{42}=0.503, P=0.001**	r_{66}=0.149, P=0.233
Pre-lay muscle mass	r_{42}=0.073, P=0.641	r_{66}=-0.114, P=0.361
Pre-lay muscle condition	r_{42}=0.040, P=0.800	r_{66}=-0.086, P=0.494
Post-lay body mass		**r_{51}=-0.326, P=0.020**
Post-lay body condition		**r_{51}=-0.361, P=0.009**
Post-lay muscle mass		r_{51}=-0.025, P=0.863
Post-lay muscle condition		r_{51}=-0.060, P=0.678
Change in muscle mass		r_{51}=0.112, P=0.435
Muscle mass change per egg		r_{51}=0.252, P=0.075
Change in body mass		r_{51}=-0.207, P=0.144
Body mass change per egg		r_{51}=-0.062, P=0.667

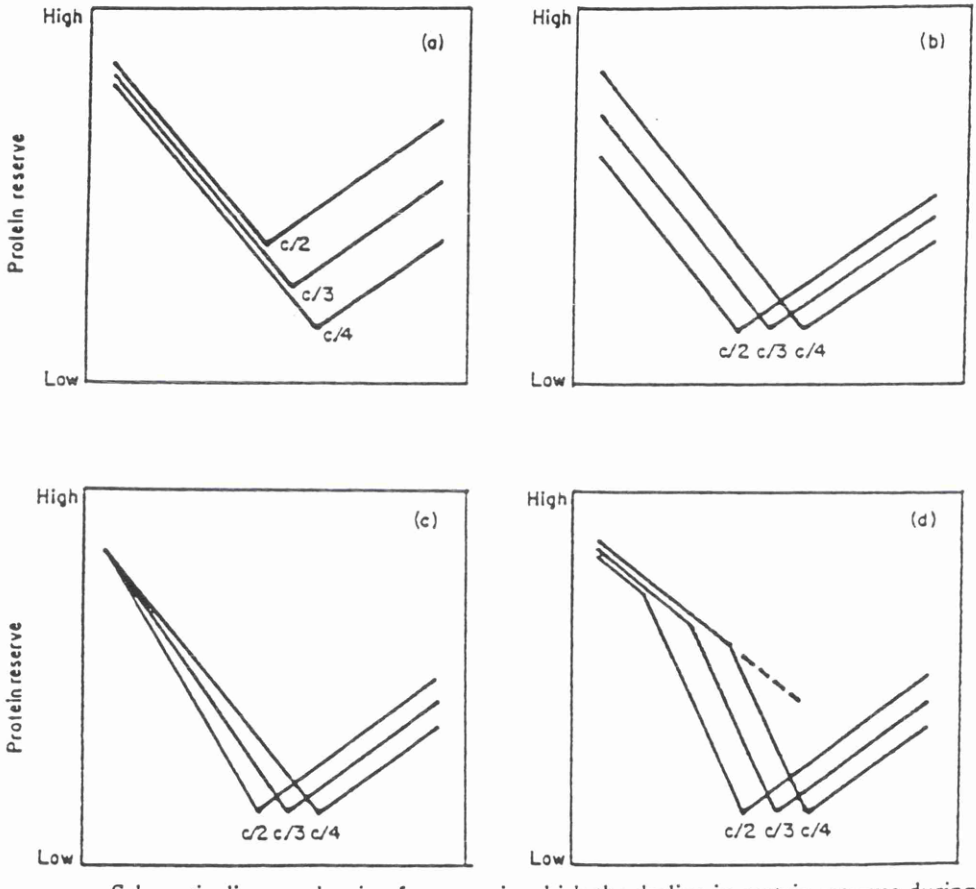
Table 3.3. Pearson's correlation coefficients for the relationships between mean egg mass and the levels of, or changes in body reserves.

	Mixed seed diet	*Panicum* diet
Pre-lay body mass	**r_{46}=0.360, P=0.014**	r_{52}=0.086, P=0.545
Pre-lay body condition	**r_{42}=0.314, P=0.043**	r_{52}=0.110, P=0.439
Pre-lay muscle mass	r_{42}=0.234, P=0.131	r_{52}=-0.029, P=0.837
Pre-lay muscle condition	r_{42}=0.081, P=0.608	r_{52}=-0.006, P=0.966
Post-lay body mass		**r_{44}=-0.419, P=0.005**
Post-lay body condition		**r_{44}=-0.340, P=0.024**
Post-lay muscle mass		r_{44}=-0.168, P=0.275
Post-lay muscle condition		r_{44}=-0.119, P=0.441
Change in muscle mass		r_{44}=-0.095, P=0.541
Muscle mass change per egg		r_{44}=-0.093, P=0.550
Change in body mass		r_{44}=-0.285, P=0.061
Body mass change per egg		r_{44}=-0.241, P=0.115

Table 3.4. Pearson's correlation coefficients for the relationships between clutch mass and the levels of, or changes in body reserves.

Diet	Mixed seed	*Panicum*
Pre-lay body mass	**r_{46}=0.464, P=0.001**	r_{52}=0.046, P=0.747
Pre-lay body condition	**r_{42}=0.503, P=0.001**	r_{52}=0.072, P=0.612
Pre-lay muscle mass	r_{42}=0.112, P=0.474	r_{52}=-0.166, P=0.240
Pre-lay muscle condition	r_{42}=0.042, P=0.791	r_{52}=-0.143, P=0.311
Post-lay body mass		**r_{44}=-0.410, P=0.006**
Post-lay body condition		**r_{44}=-0.404, P=0.007**
Post-lay muscle mass		r_{44}=-0.085, P=0.582
Post-lay muscle condition		r_{44}=-0.072, P=0.645
Change in muscle mass		r_{44}=0.021, P=0.894
Muscle mass change per egg		r_{44}=0.096, P=0.533
Change in body mass		r_{44}=-0.235, P=0.125
Body mass change per egg		r_{44}=-0.130, P=0.401

Figure 3.1



Schematic diagram showing four ways in which the decline in protein reserves during egg production might determine the number of eggs laid. The range of clutch-sizes included has been restricted to two, three and four eggs, though in Scheme (d) the dotted line (the minimal possible rate of decline), if continued, would lead to a clutch of six or seven.
(From Jones and Ward, 1976)

Figure 3.2a. Regression of body mass on body size. The residuals give "body condition".
y = 14.72032 + 0.59174x

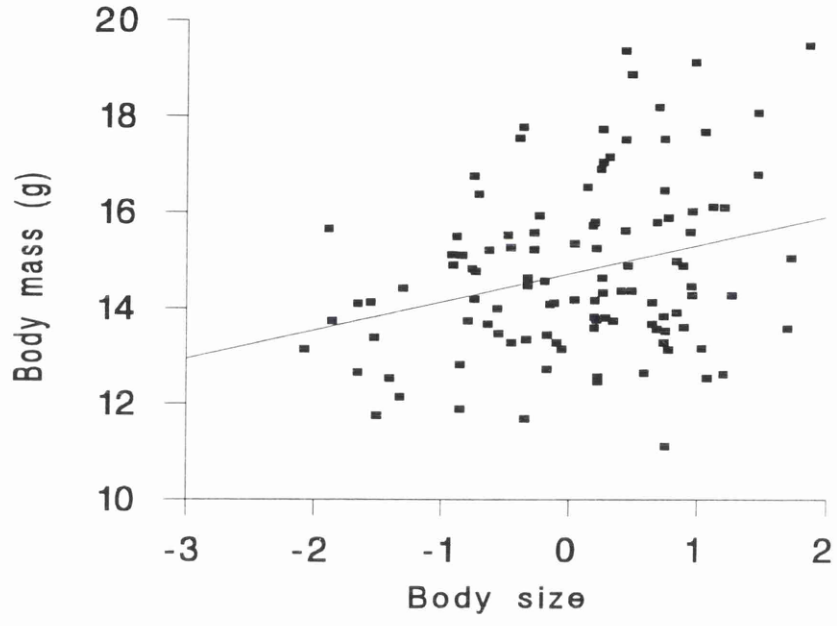


Figure 3.2b. Regression of muscle mass on body size. The residuals give "muscle condition".
y = 0.73575 + 0.03336x

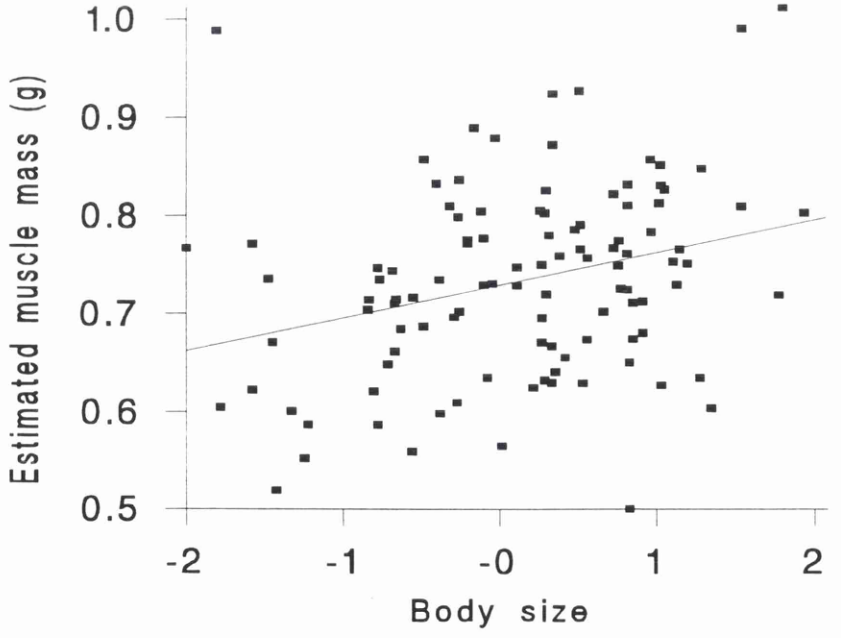


Figure 3.3. Correlation between the number of days between pairing and laying and female body mass at the time of pairing. Spearman's r=-0.224, n=114, P=0.017.

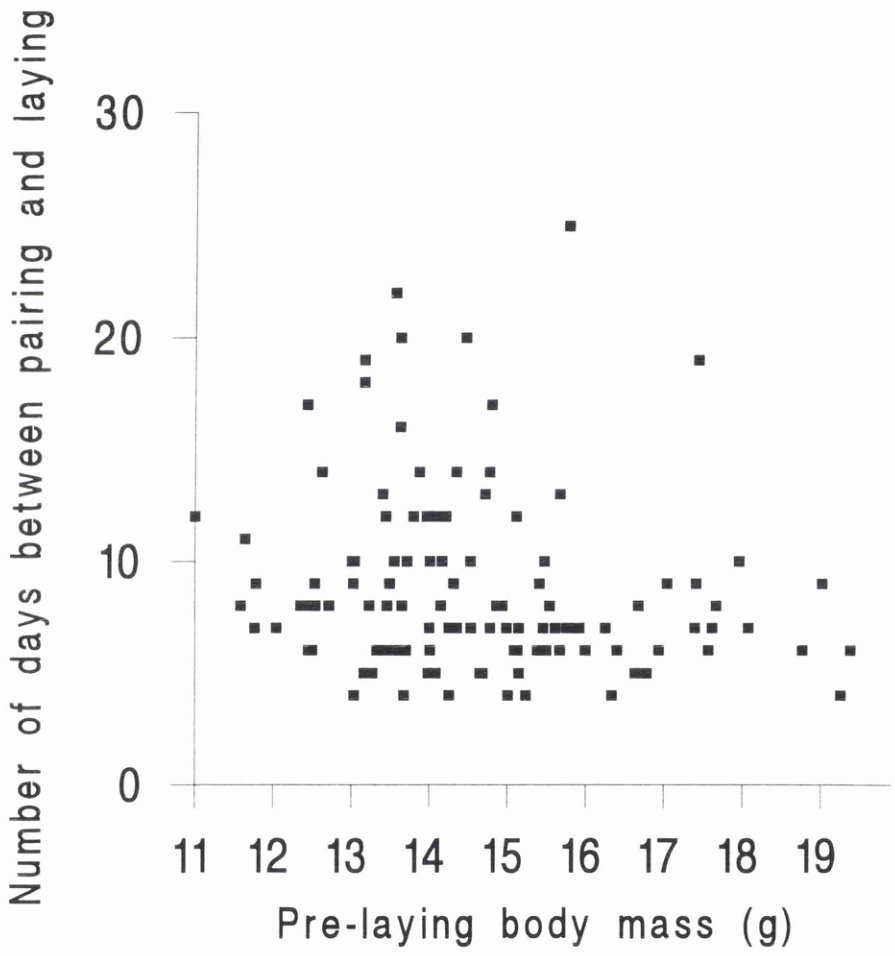


Figure 3.4. Correlation of the number of days from pairing to laying and pre-laying body condition. Spearman's r=-0.278, n=108, P=0.004.

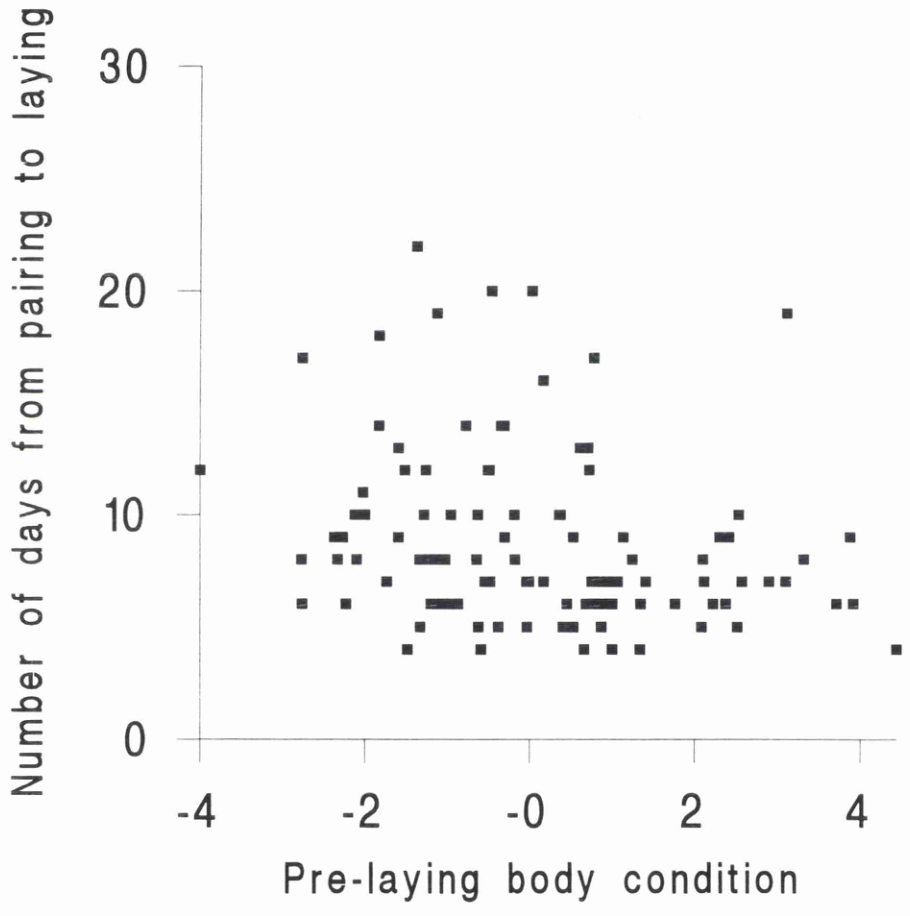


Figure 3.5. Correlation between estimated pectoral muscle lean, dry mass at pairing and the number of days between pairing and laying. Spearman's r=-0.010, n=108, P=0.920.

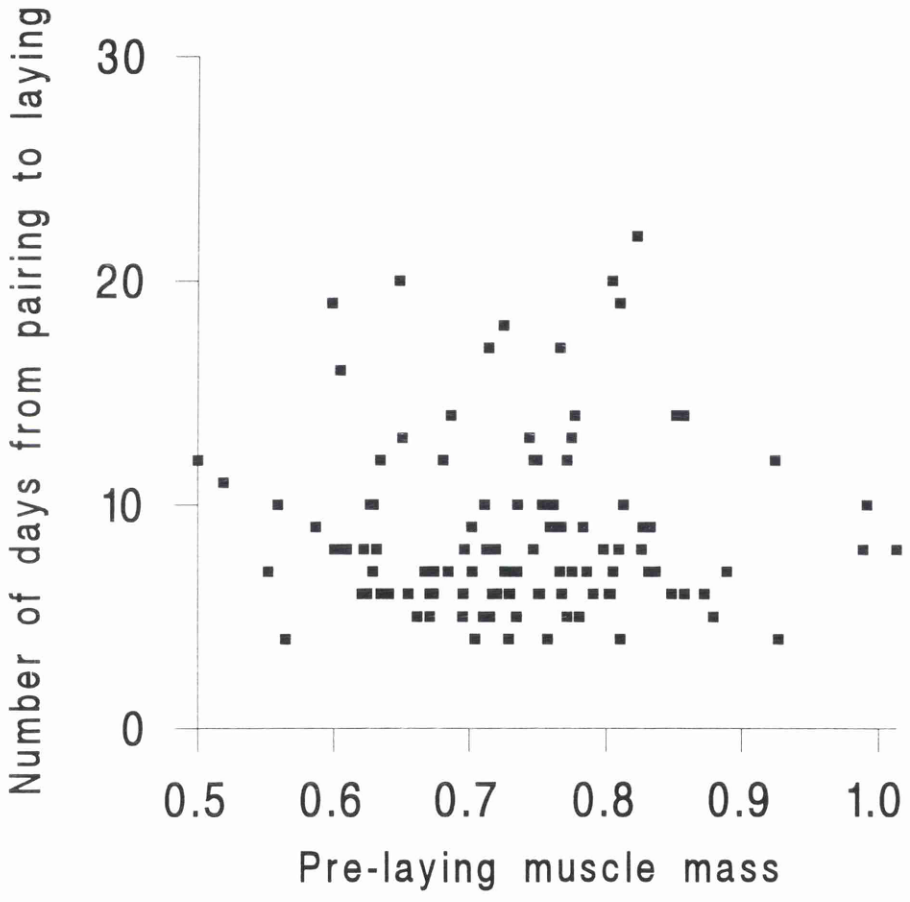


Figure 3.6. Correlation between pre-laying muscle condition and the number of days between pairing and laying. Spearman's r=-0.015, n=108, P=0.874.

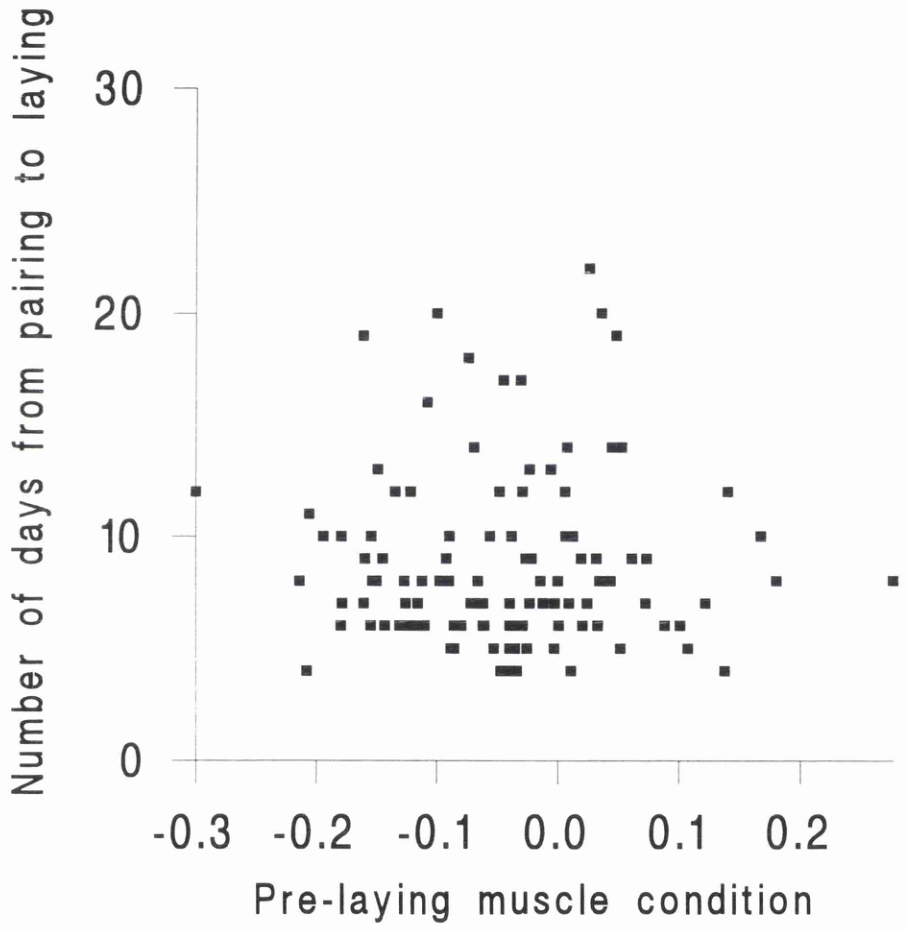


Figure 3.7. Correlation of pre-laying body mass and clutch size for birds on a diet of mixed seed. Spearman's r=0.442, n=48, P=0.002.

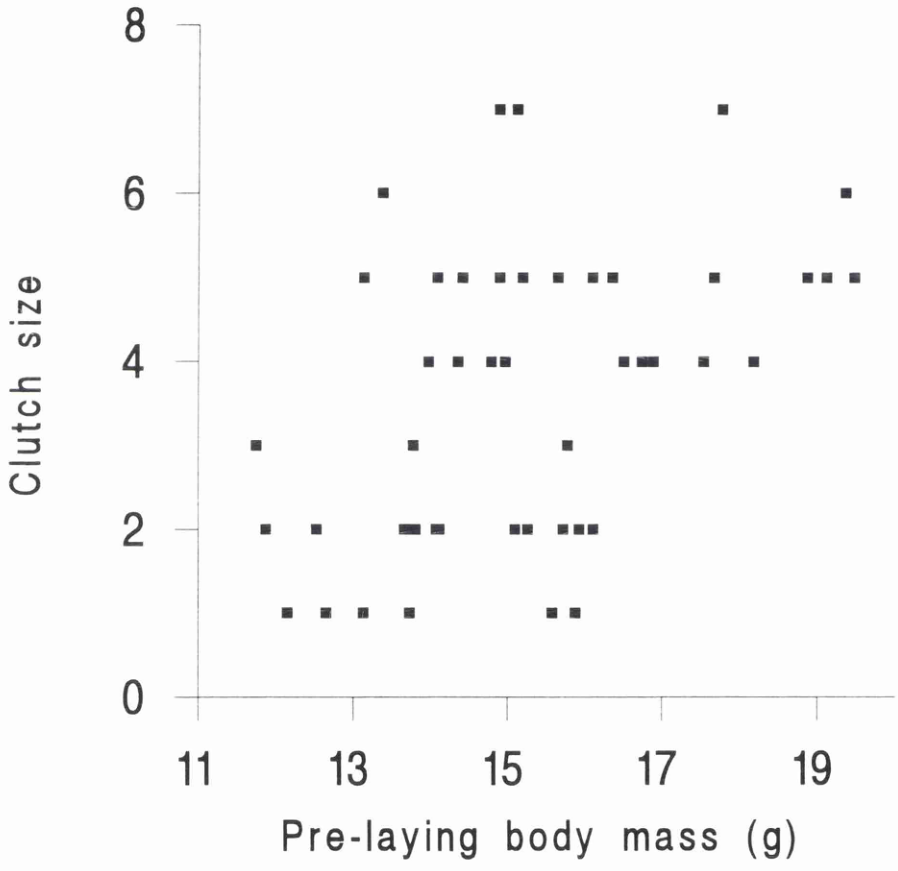


Figure 3.8. Correlation of clutch size and pre-laying body condition for birds on a diet of mixed seed. Spearman's r=0.503, n=42, P=0.001.

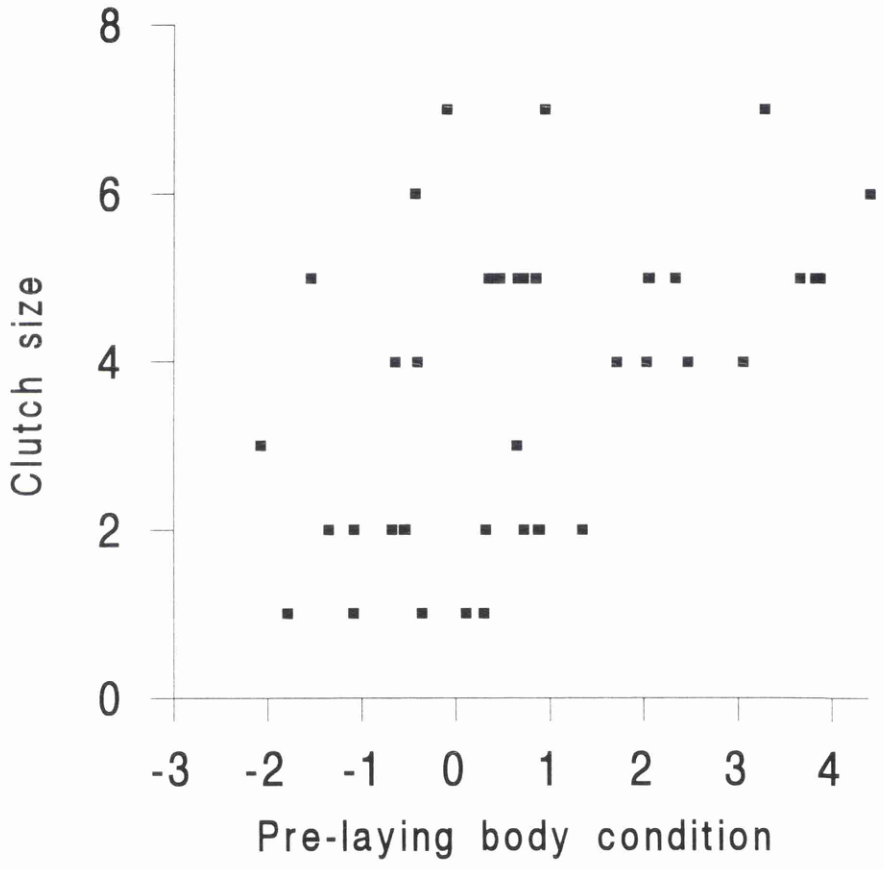


Figure 3.9. Correlation of post-laying body mass with clutch size for birds on a diet of Panicum millet. Spearman's r=-0.326, n=51, P=0.020.

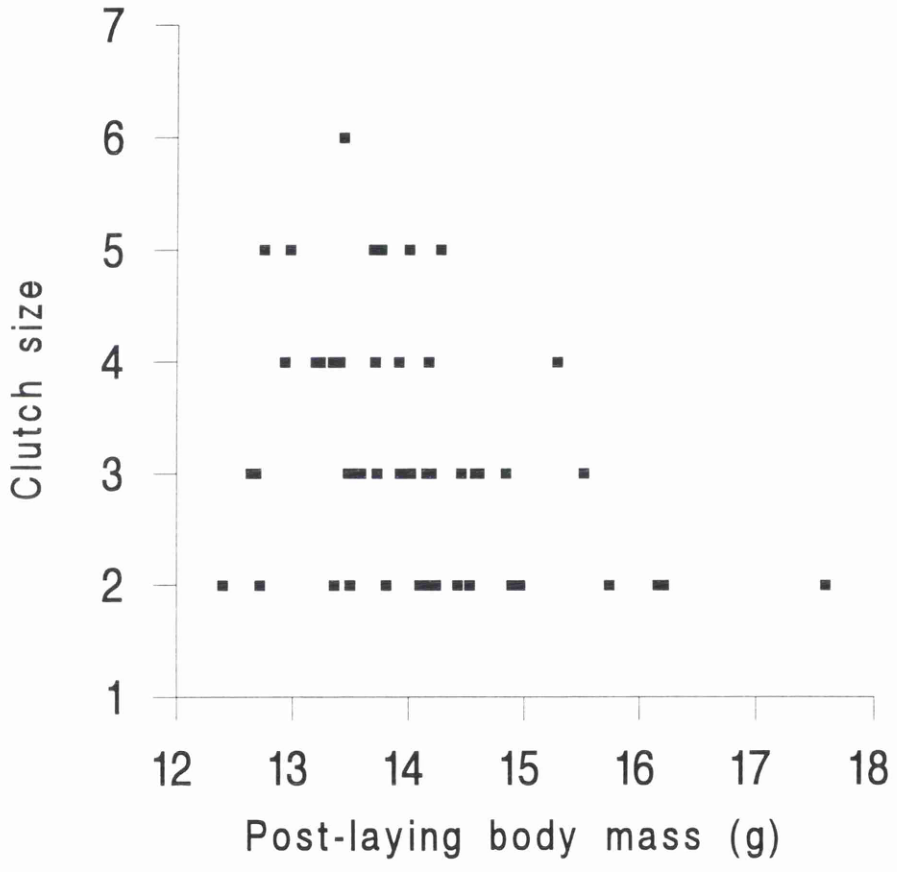


Figure 3.10. Correlation of clutch size and post-laying body condition for birds on a diet of Panicum millet. Spearman's r=-0.361, n=51, P=0.009.

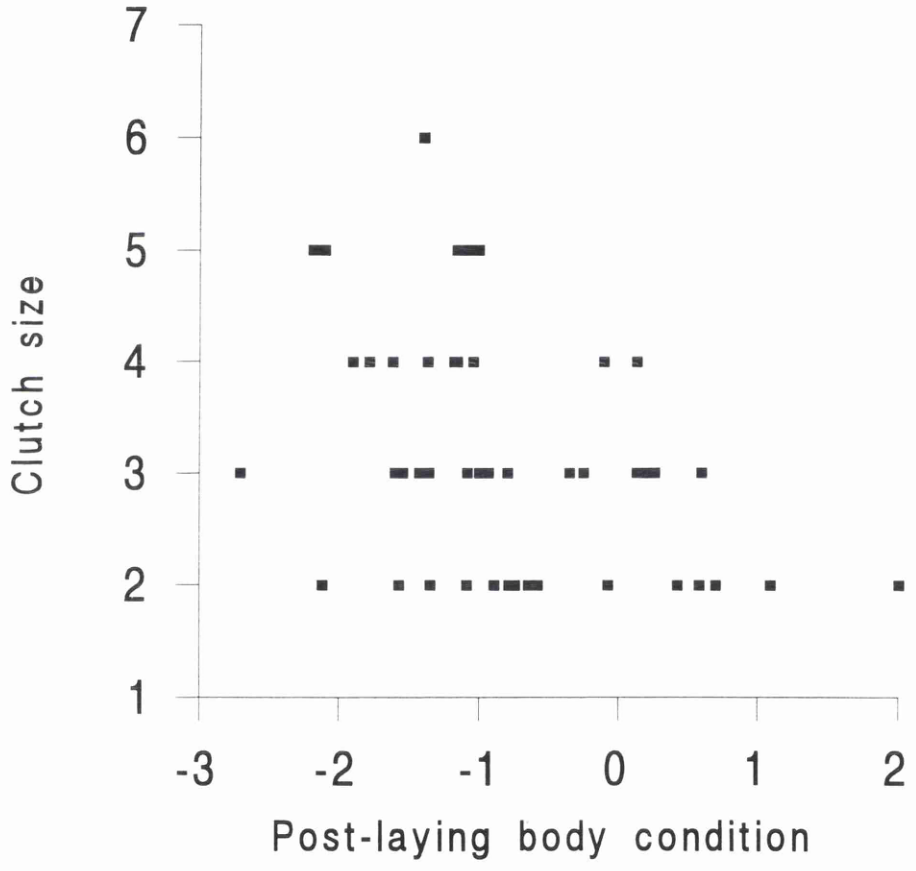


Figure 3.11a. Correlation of clutch size and the number of days between pairing and laying for birds on a diet of mixed seed. Spearman's r=-0.366, n=49, P=0.010.

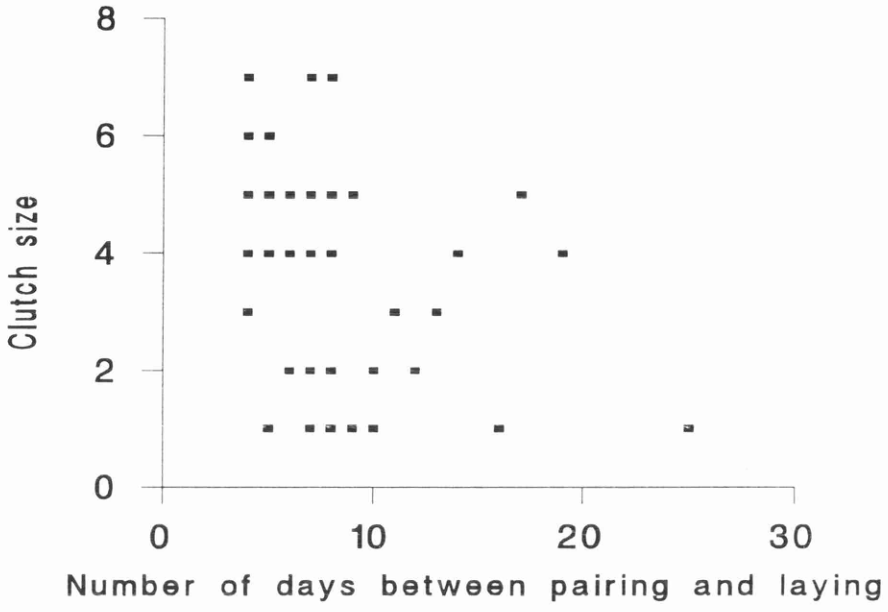


Figure 3.11b. Correlation of clutch size and the number of days between pairing and laying for birds on a diet of Panicum millet. Spearman's r=-0.348, n=66, P=0.004.

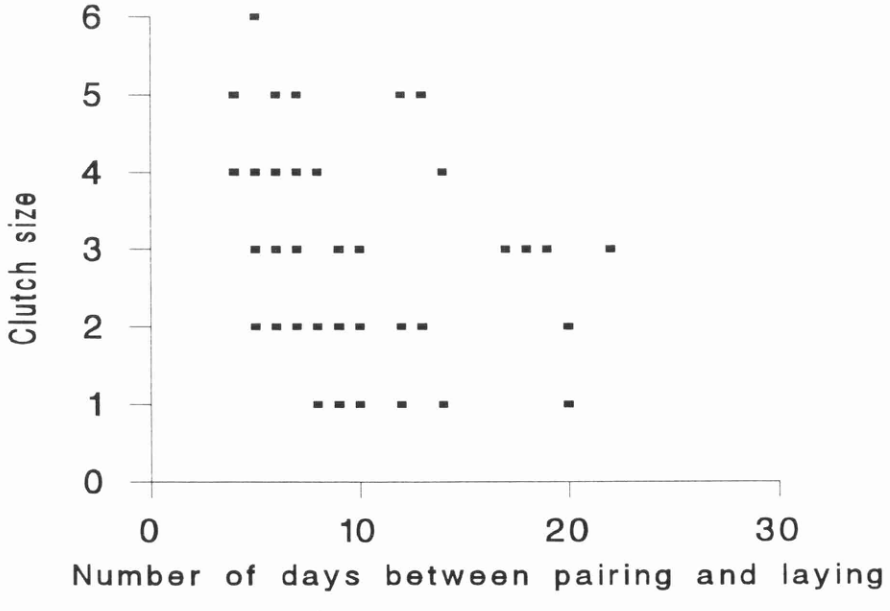


Figure 3.12. Correlation of mean egg mass and female body mass at the time of pairing for birds on a diet of mixed seed. Pearson's r=0.360, n=46, P=0.014.

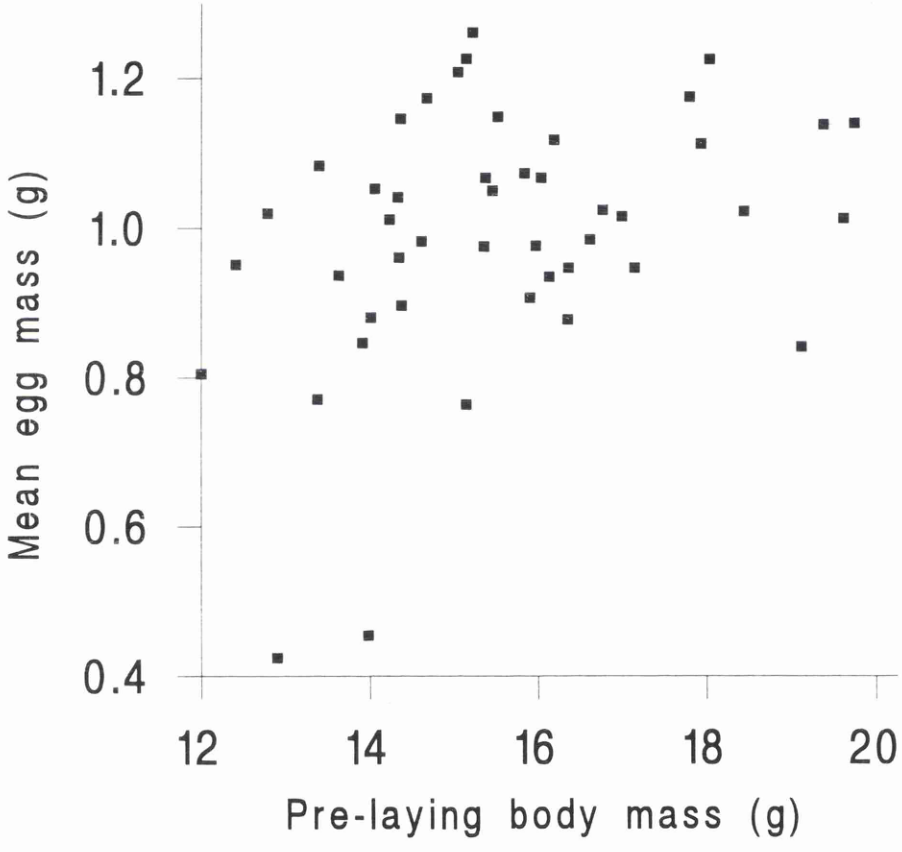


Figure 3.13. Correlation of mean egg mass and body condition at the time of pairing for birds on a diet of mixed seed. Pearson's r=0.314, n=42, P=0.043.

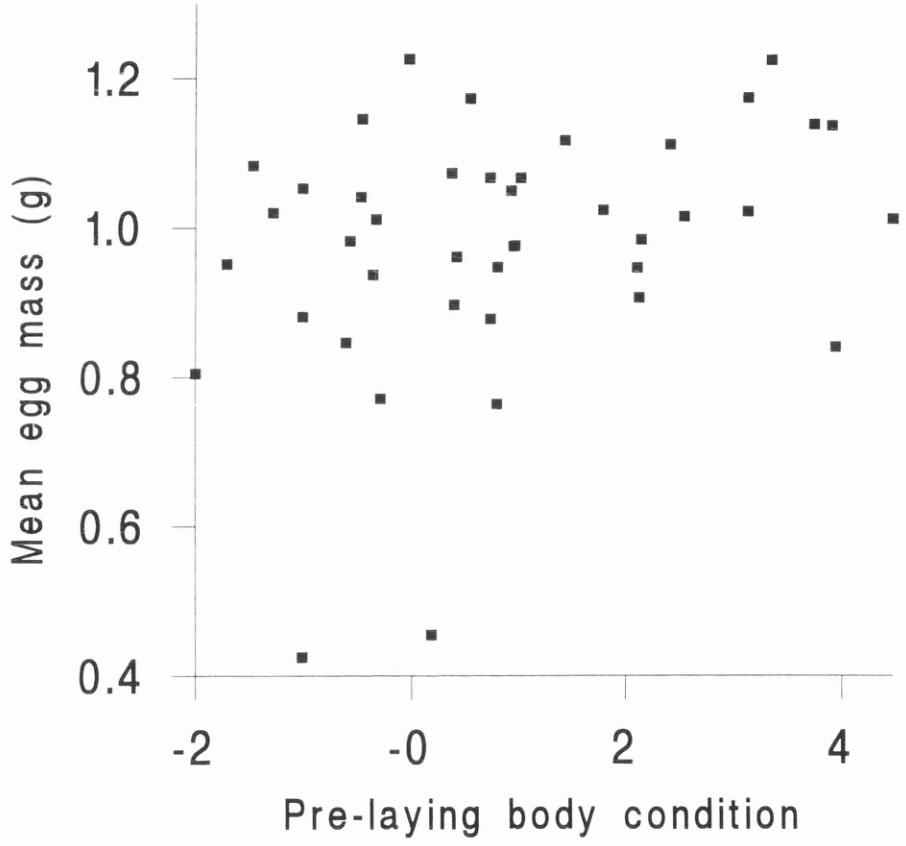


Figure 3.14. Correlation of mean egg mass and post-laying body mass for birds on a diet of Panicum millet. Pearson's r=-0.419, n=44, P=0.005.

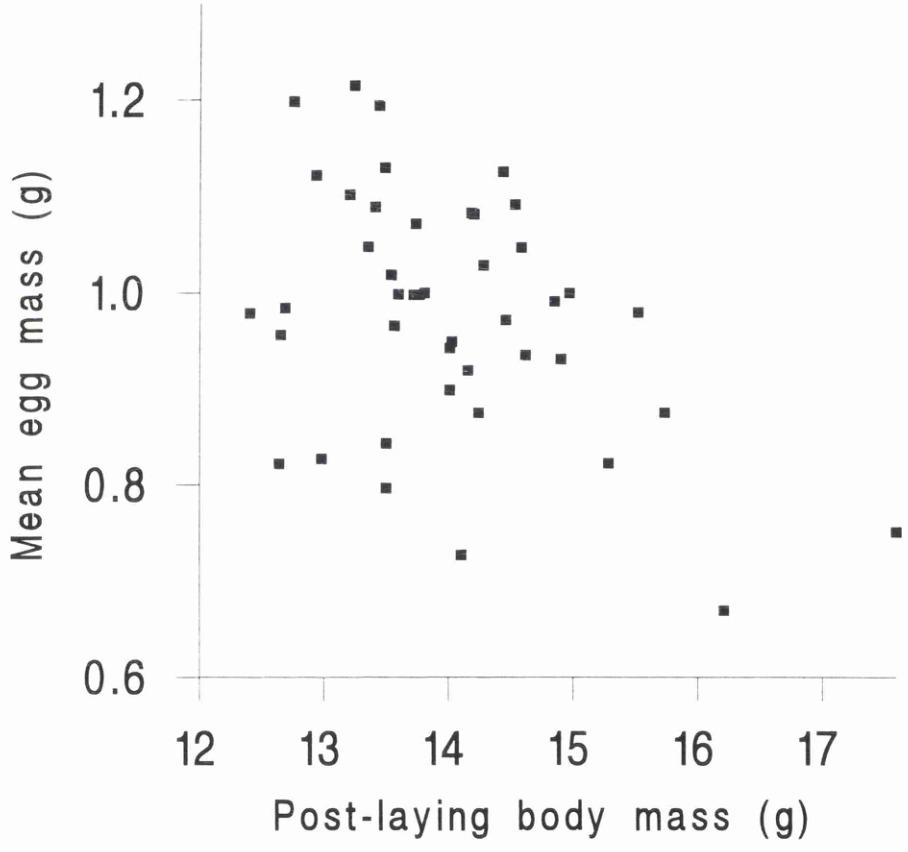


Figure 3.15. Correlation of mean egg mass and post-laying body condition for birds on a diet of Panicum millet. Pearson's r=-0.347, n=44, P=0.021.

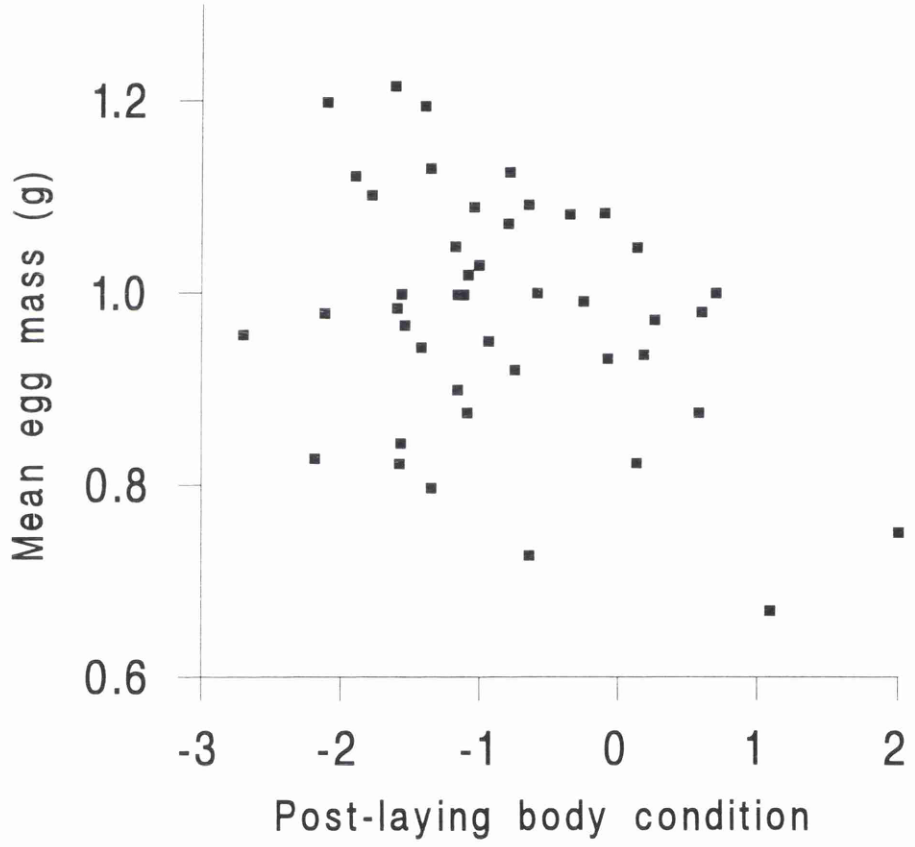


Figure 3.16. Correlation of clutch mass and pre-laying body mass for birds on a diet of mixed seed. Pearson's r=0.464, n=46, P=0.001.

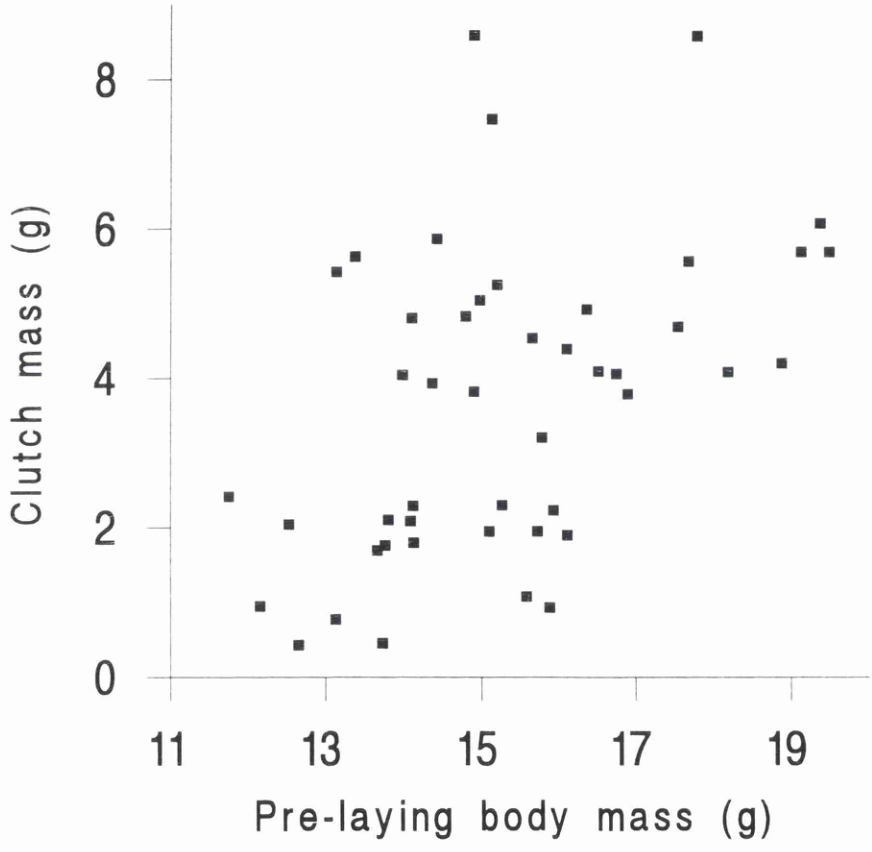


Figure 3.17. Correlation of clutch mass and pre-laying body condition for birds on a diet of mixed seed. Pearson's r=0.503, n=42, P=0.001.

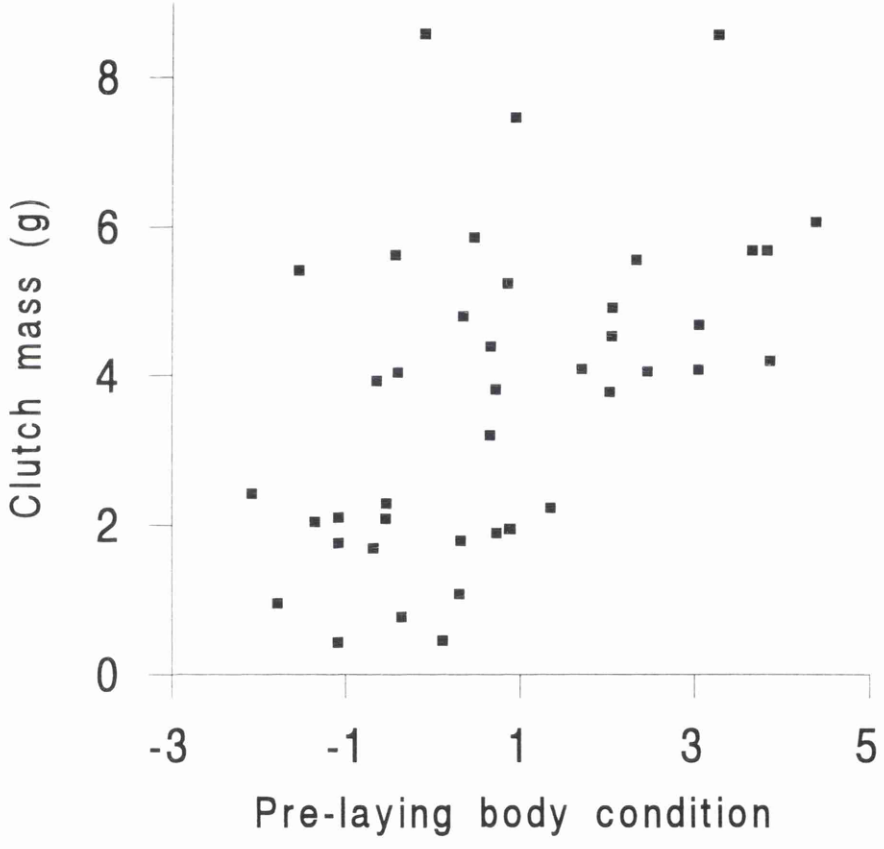


Figure 3.18. Correlation of clutch mass and post-laying body mass for birds on a diet of Panicum millet. Pearson's r=-0.410, n=44, P=0.006.

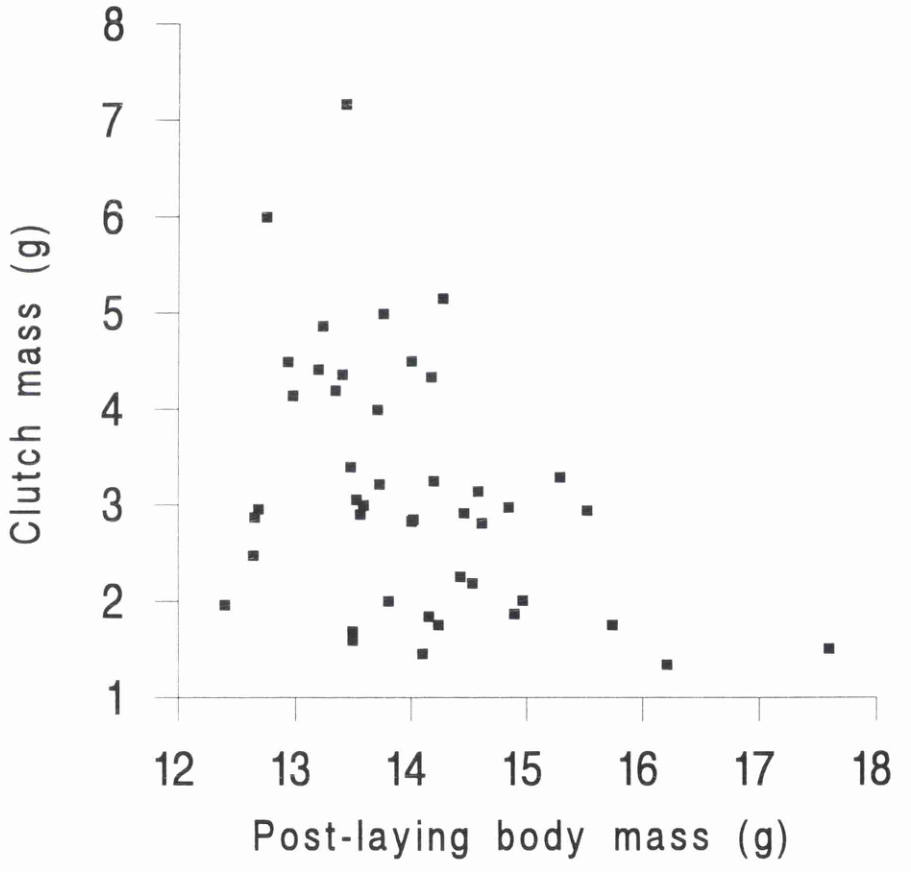


Figure 3.19. Correlation of clutch mass and post-laying body condition for birds on a diet of Panicum millet. Pearson's r=-0.404, n=44, P=0.007.

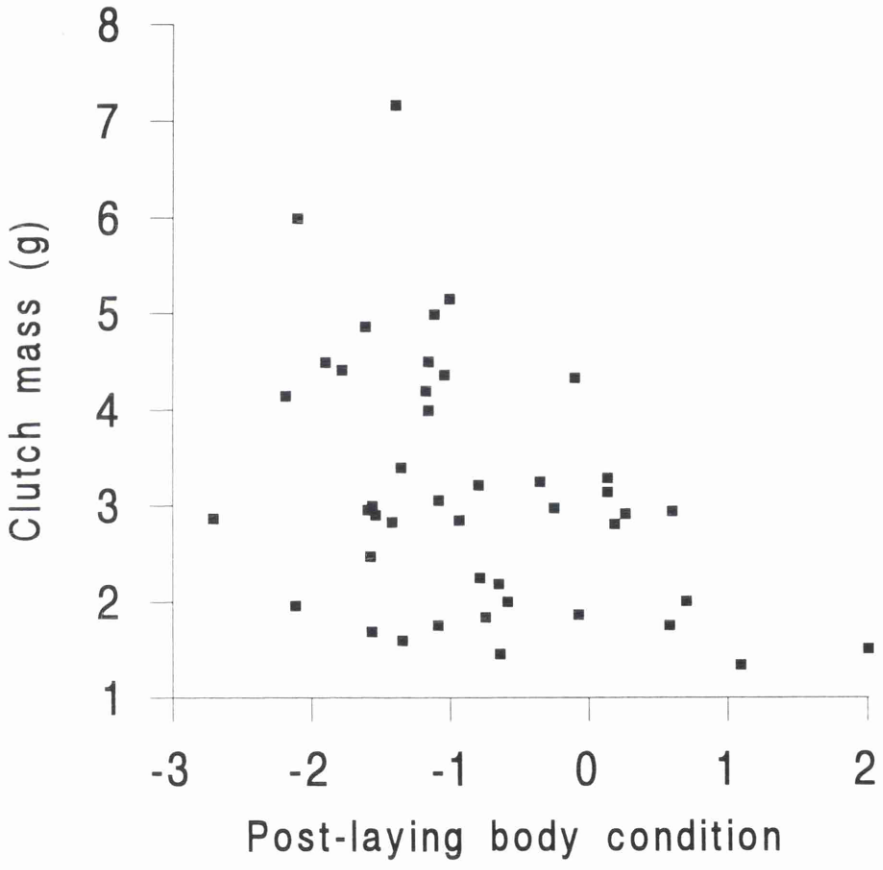


Figure 3.20. Correlation of clutch size and rate of seed intake during the period of egg formation. Spearman's r=-0.469, n=63, P<0.001.

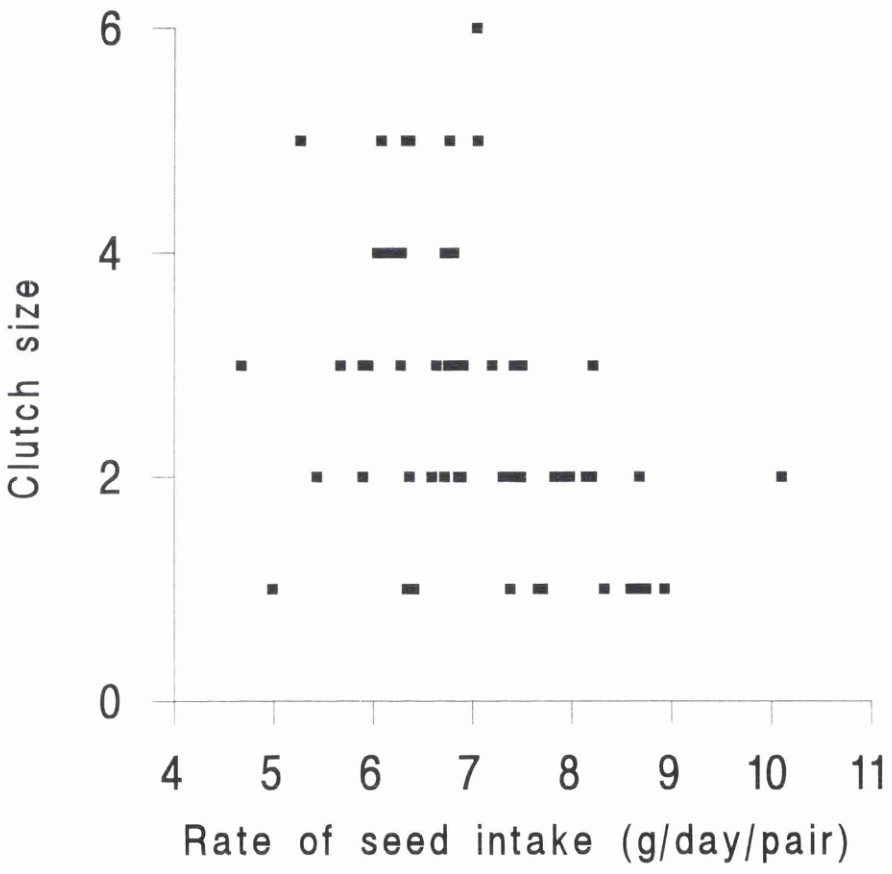


Figure 3.21. Correlation of mean egg mass and rate of seed intake during the period of egg formation. Pearson's r=-0.290, n=50, P=0.041.

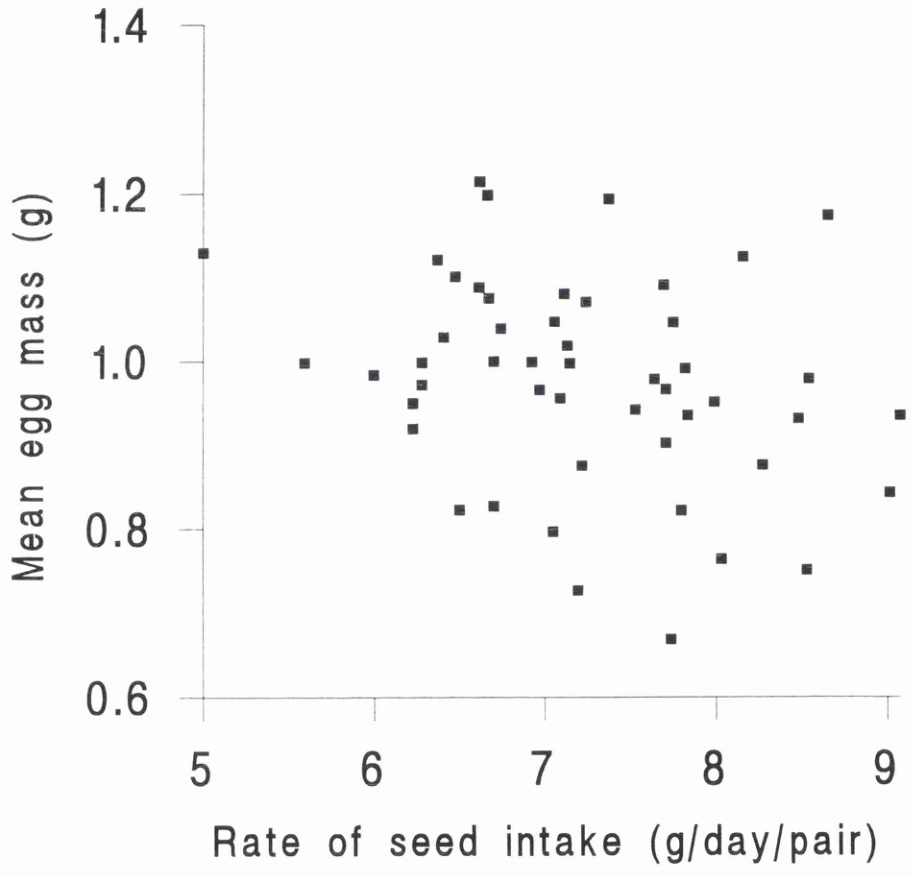


Figure 3.22. Correlation of the change in female body mass and the rate of seed intake during the period of egg formation. Pearson's r=0.464, n=49, P=0.001.

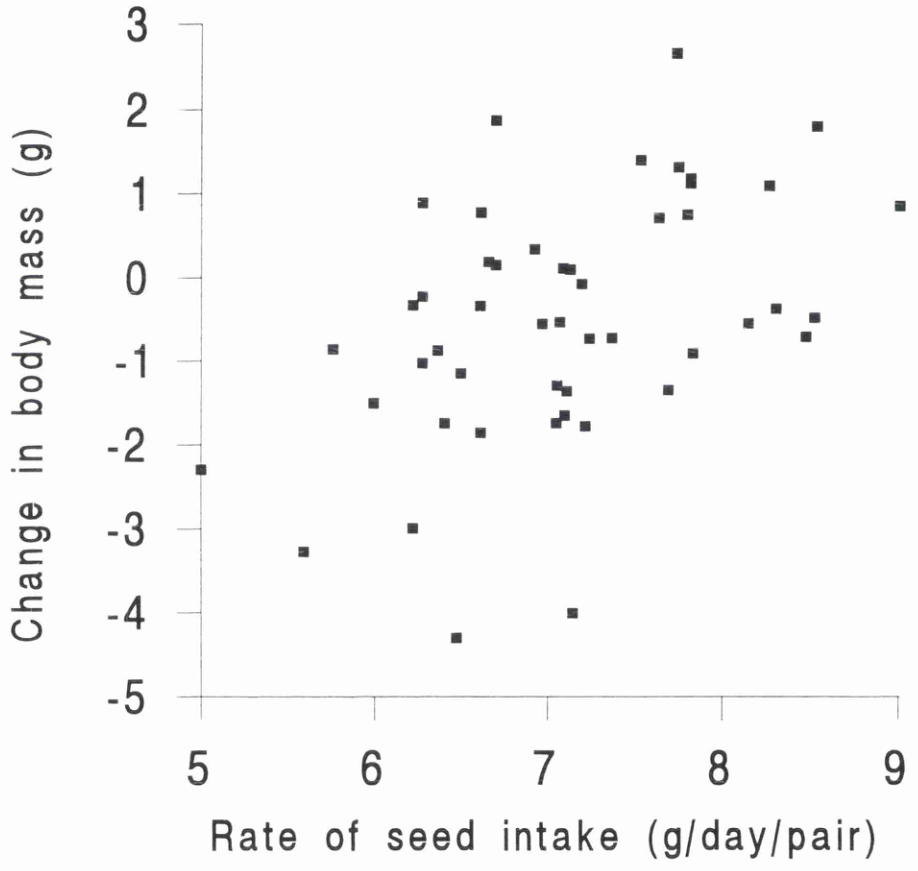


Figure 3.23. Correlation of post-laying body mass and the rate of seed intake during the period of egg formation for birds on a diet of Panicum millet. Pearson's r=0.397, n=49, P=0.005.

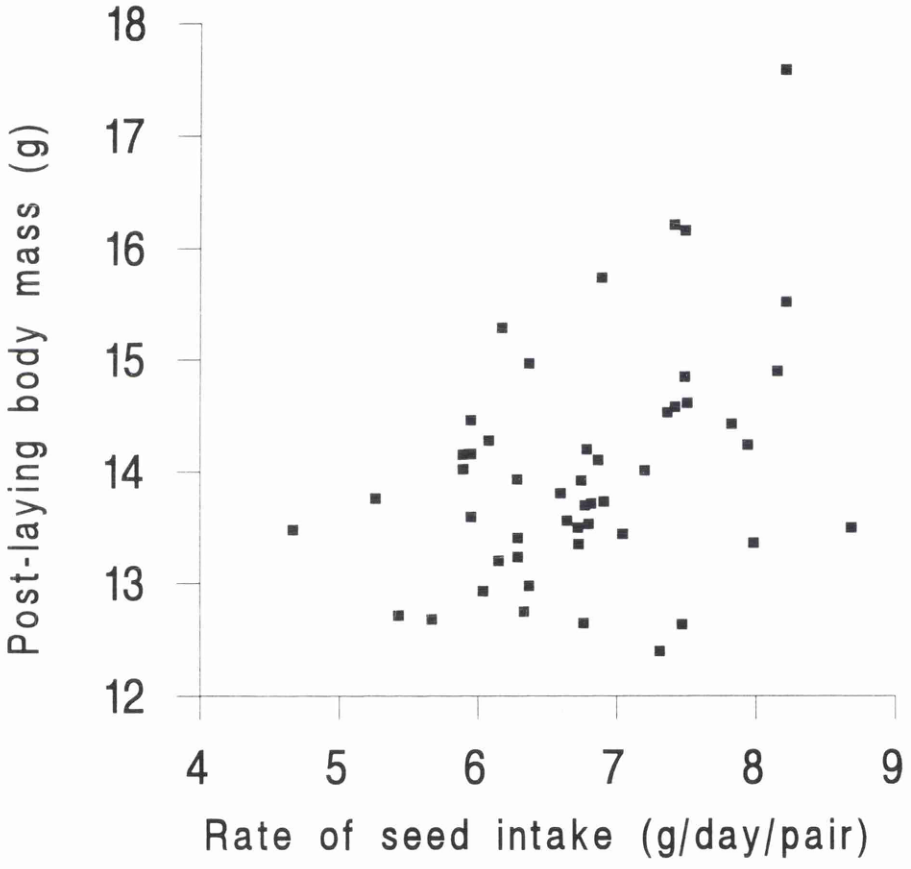


Figure 3.24. Correlation of post-laying body condition and the rate of seed intake during egg formation for birds on a diet of Panicum millet. Pearson's r=0.374, n=49, P=0.008.

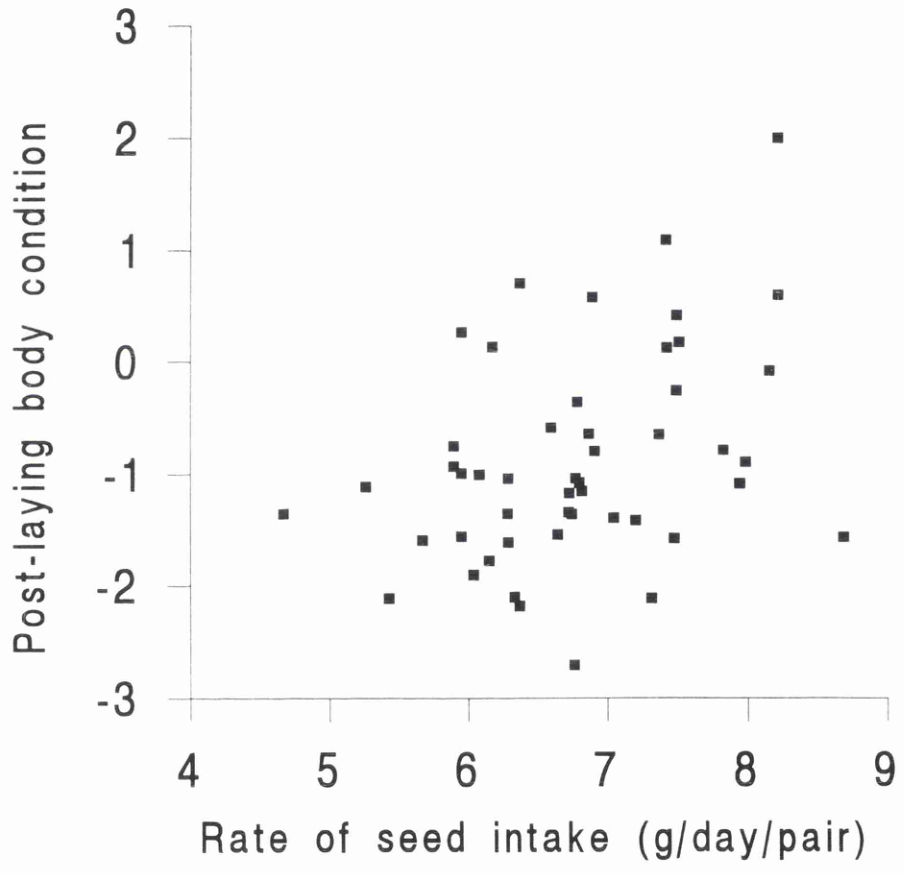


Figure 3.25. Correlation of muscle condition at pairing with the change in muscle mass during egg formation. Pearson's r=-0.501, n=51, P<0.001.

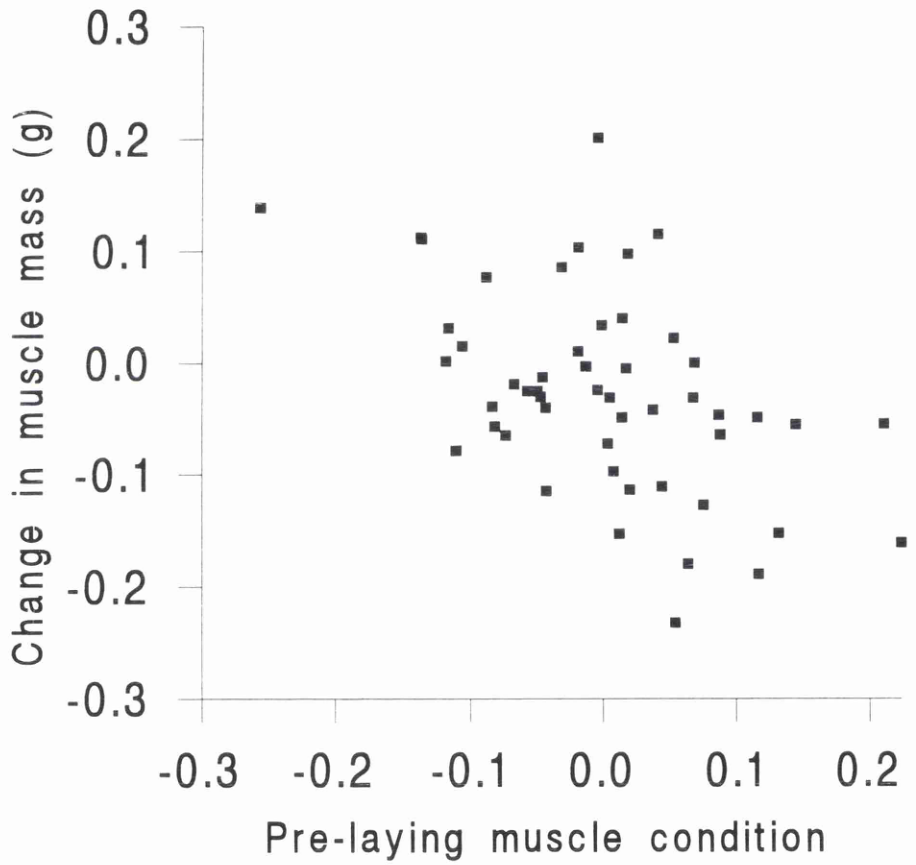


Figure 3.26. Correlation of muscle condition following laying with the change in muscle mass during egg formation. Pearson's r=0.497, n=51, P<0.001.

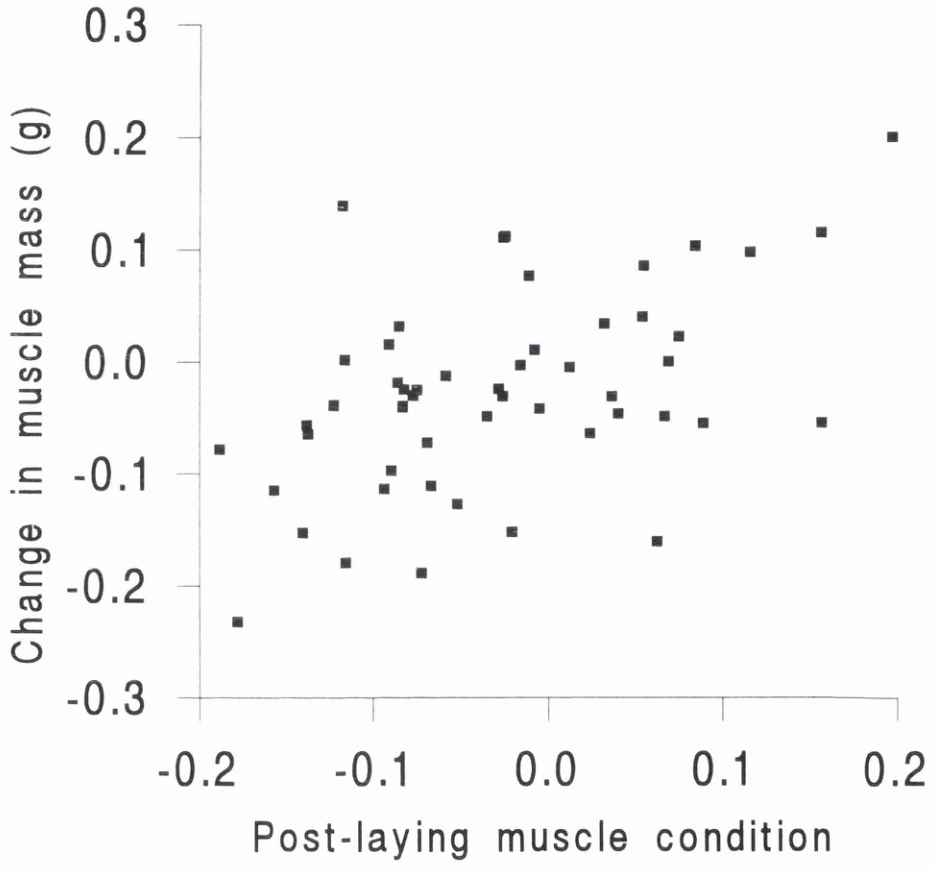
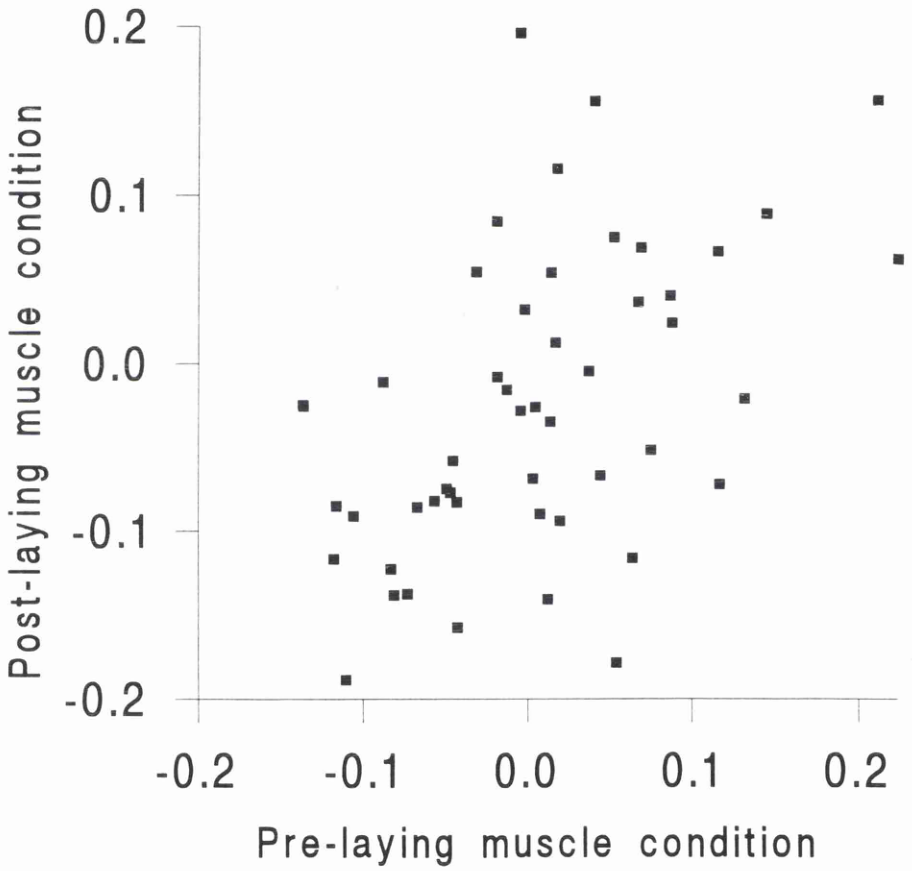


Figure 3.27. Correlation of pre-laying and post-laying muscle condition. Pearson's r=0.502, n=51, P<0.001.



Discussion

Body reserves and breeding

Field studies of the zebra finch have not considered the potential effect of protein condition or body condition on breeding. Davies (1977) noted that in a year when the winter masses of birds were relatively low there were few young fledged. The number of breeding birds, however, was not recorded. There are no comparable values for body and muscle condition, or muscle mass, in wild zebra finches, but body masses have been recorded in the wild, with most birds between 11g and 13g (Davies, 1977), and for captive, wild-caught birds, mostly between 10g and 12g (Zann and Straw, 1984).

Condition could affect the timing of breeding if it provides a threshold for breeding to occur, or for the production of a particular clutch size (Jones and Ward, 1976; Drent and Daan, 1980). In this study of captive birds, body condition was higher in breeders than non-breeders, indicating that birds may require good body condition to breed. Also, birds above a threshold of body condition laid very soon after pairing, whereas below this threshold, the timing of laying was related to the level of body condition at pairing, the birds with the poorest body condition delayed laying for the longest. There were no such relationships for the level of protein condition, as suggested for the red-billed quelea (Jones and Ward, 1976). This indicates that either the level of body fat, or the overall body condition (protein and fat status, together) may act as a physiological control variable, or as a cue for the control of breeding.

Most of the birds laid very soon after pairing. Because they have evolved as opportunistic breeders, reacting quickly to an improvement in environmental conditions in an unpredictable, arid environment, they could therefore be expected to lay quickly after pairing, though there is evidence here of some delay in laying by

some birds. These late-layers tended to lay small clutches. This could be interpreted as equivalent to the seasonal decline in clutch size found for many temperate birds. Late-layers may face a less predictable supply of food, after a peak of food availability in the desert. A bird in poor condition might increase its potential clutch size by delaying laying to increase its body reserves, these being the main source of egg proteins (Houston, Donnan and Jones, in press *a*). However, on the low protein diet of *Panicum* millet provided after the birds had been paired, there would have been very little opportunity to increase their protein reserves by delaying the date of laying. Birds that delayed laying may therefore not have increased their potential clutch sizes and so laid relatively small clutches.

Body reserves and egg production

The models of Jones and Ward (1976) were tested by comparing the size of the protein reserves prior to and following egg production with the clutch size produced. No relationship was found between the loss of protein condition and clutch size, or even mean egg mass or clutch mass. Therefore the birds may all have lost similar amounts of protein regardless of the number of eggs laid. Protein reserves are certainly important in egg production: Houston, Donnan and Jones (in press *a*) have shown that a majority of the proteins in a clutch of zebra finch eggs come from the body reserves of the laying female. The rate of protein loss, not the quantity of protein lost, may therefore affect egg production. This fits models (c) and (d), but not (a) or (b). If this is the case then the amount of protein lost per egg should be negatively related to clutch size. In fact there was no significant correlation, and the trend was for slightly more protein loss per egg, not less, with increasing clutch size. Daily changes in protein condition cannot be determined using the pectoral muscle moulding technique: it is unlikely to be accurate enough. Daily changes in mass can be recorded by weighing, but the use of reserves cannot, since overall body weight will increase as the reproductive tissues and ova develop, and decrease as they are lost.

Changes in body mass therefore do not solely reflect changes in body reserves during egg formation itself. They can only be compared before and after this period.

One explanation might be that losses from the pectoral muscles do not reflect the changes in body protein levels. This is unlikely since Houston, Donnan and Jones (in press *a*) have shown that proportionally more protein was lost from the pectoral muscles of their zebra finches than was expected from their proportion of body mass. They are therefore possibly the best site at which to monitor changes in protein condition (see chapter 2, introduction).

Any relationship between protein reserves and egg production could be masked by variations in protein excretion. All of the birds may have used their protein reserves to a similar degree, but some may have been unable to use all of the protein released. This implies an effect of reserve quality, not just the size of a reserve. Birds with the same levels of body protein may differ greatly in the amount of protein usable in egg production. This potential effect could be tested by providing diets of differing protein quality prior to egg production, then allowing all of the birds to breed on a basic diet. Differences in egg production may then reflect variation in the size of protein reserves, measured by pectoral muscle profiling (chapter 2), or by the quality of the protein reserves, determined by pre-breeding diet (chapter 4).

Alternatively, fat may be more important than protein in the control of egg production. An analysis of the relationships between body condition and egg production reveals that pre-laying body condition correlates with clutch size, mean egg mass and clutch mass, but only on the mixed seed diet. This raises the possibility of a model for fat reserves, like model (b), of Jones and Ward, for protein reserves. On the *Panicum* diet, only post-laying body condition related to egg production, not pre-laying body condition. Body reserves might therefore follow a model similar to model (a). However, the overall change in body mass did not relate to egg

production. This is surprising: I would have expected a relationship if body condition followed a model similar to either (a) or (b). If the rate of use of the fat stores affected the number of eggs that could be laid by a bird with limited fat stores, then there should have been a relationship between the change in mass per egg laid and the number of eggs that were produced. This was not the case, but clutch size, mean egg mass and clutch mass all related to the body masses of females following laying. This gives a stronger correlation than that for body condition, indicating that there may be an effect which is due to the size of the body reserves of a female, but is unaffected by her body size. Similar correlations were not found for muscle mass or muscle condition, so I deduce that these effects may be due to the status of fat reserves, rather than protein. The birds therefore completed laying with energy reserves that are in proportion to the number of eggs that they laid. This might leave sufficient reserves to cover another stressful period soon after, i.e. either the incubation period or chick rearing. There is some evidence that incubation can be costly to a bird (Taborsky and Brugger, 1994), as can chick rearing (Johnston, 1993) due to the limited time available for feeding. In consequence there can be a reduction in body mass. If the size of a bird's body reserves can limit the survival of its young or its future reproductive potential, then there should be selection to invest as much of its reserves as would allow for the maximum number of offspring to survive to reproductive age, whilst making sure that an adequate level remains within the body to complete the rearing of the young and enable the parent to survive into the next season.

Inaccuracies in the estimation of muscle mass could have blurred the results if the errors are great in comparison with the observable changes in mass. However, Houston, Donnan and Jones (in press *a)* showed a 14% loss of muscle mass during egg production. An assessment of the accuracy of the protein estimation technique (chapter 2) shows a mean 8% difference between estimated and actual values. This should be adequate to show whether significant losses occurred, but may leave a substantial margin of error that might blur any trends in the results. In chapter 4,

however, there is evidence that the technique is accurate enough to show such trends in the data. My estimates of muscle condition have so far not proved to be highly accurate. This might explain such inconclusive results. The estimates, however, do correlate with the actual values (chapter 2).

It is possible that by measuring the size of pectoral muscles I have not measured the amount of reserve protein usable during egg production. The quality of a protein reserve might affect this. The next step was therefore to look at the possibility of a protein quality effect in order that any relationships between egg production and the use of protein reserves can be better elucidated.

Food intake and the use of body reserves.

During egg formation food intake might be expected to increase, to allow for the greater nutritional demands on a zebra finch at this time, as shown by the losses of both fat and protein (Houston, Donnan and Jones (in press *a*). This was found to be the case by El Wailly (1966) in a study of zebra finches, but Houston, Donnan and Jones found no difference in seed consumption between laying and non-laying pairs. This was not due to a "digestive bottleneck" (the limited ability of birds to digest more than a certain mass of food in daylight hours) since longer daylight hours did not lead to a greater food intake.

In this study, the results were different again, and at first appeared very puzzling. Breeding pairs had a lower daily seed intake than non-breeding pairs, although a surplus of food was provided. In fact, seed intake was negatively related to clutch size: the largest clutches had the lowest seed intakes during the period of egg formation. There was also an apparent negative relationship between seed intake and mean egg mass, but it was weak and unconvincing. Changes in body masses were

related to seed intake: females from low intake pairs lost relatively large amounts. No relationship was found between changes in protein condition and daily seed intakes.

Why should birds eat less food during egg production? Firstly, a reduction in activity has been observed at this time in wild willow flycatchers, *Empidonax trailli*, (Ettinger and King, 1979), wild *Camaroptera* (Fogden and Fogden, 1979), and captive zebra finches (Houston, Donnan and Jones, in press *a*). It is not known why this occurs. It could be due to the fragility of the eggs during their production (Fogden and Fogden, 1979). Alternatively it may be due to the increase in the weight of females as a result of the build up of the reproductive organs and body reserves prior to the laying of the first egg. This might increase the energy used in flight, reducing the pay-off to searching for food and increasing the risk of predation whilst the manoeuvrability and speed of flight may be reduced. A reduction in food intake might, secondly, be required to trigger a physiological response within the body, such as the release of reserves. On the other hand, the release of reserves may occur due to a reduction in the concentrations of particular nutrients in the blood as a result of their use in egg formation. These may be specific nutrients that are lacking in sufficient quantities in the diet to support egg formation. If these are found in low concentrations in body tissues, then substantial breakdown of tissues may be required in order to release enough to prevent the limitation of egg production. Not all of these nutrients may be absorbed quickly from the blood, so blood levels of many nutrients may remain high for long periods. This might reduce a bird's hunger for low quality food, such as *Panicum* millet. This seed might only serve to boost the levels of the non-limiting nutrients.

Some essential amino acids, such as the sulphur amino acids, cysteine and methionine, are found in egg proteins in greater quantities than in plant proteins and some animal proteins (Harvey, 1970). The ability to sequester these over the period leading up to egg formation, and to release them quickly, could therefore be of great value.

Houston, Donnan and Jones (in press *a*) have shown that zebra finches will breed when the amino acid requirements of the eggs are not in balance with the dietary intake.

In summary, these results are difficult to interpret. We know that zebra finches rely substantially on endogenous protein for egg production. It is reasonable therefore to expect strong correlations between female muscle condition and egg production. No such associations were detected using the pectoral muscle profiling technique. There was no evidence for a threshold level of protein for breeding to occur, or for a range of thresholds relating to the clutch size that will be laid. Neither was there evidence for any control of egg production by the levels of protein reserves. I therefore conclude that breeding was not limited by the levels of general protein reserves. The levels of body condition (including fat reserves), however, appear to relate to the timing of laying, and birds seem to control the loss of these reserves in order to prepare for their use during the incubation or rearing periods. A majority of the egg proteins come from body reserves (Houston, Donnan and Jones, in press *a*) so I am inclined to think that muscle condition may affect egg formation, but there is no simple relationship between the two. Differences in the diets prior to breeding could have influenced egg formation through effects on the quality of body reserves. The birds were placed under a variety of different dietary regimes prior to pairing in order to produce a wide range of muscle condition in the experimental birds. This may have introduced a great deal of variation to the data set that was not accounted for by differences in the size of the protein reserves or in body mass, i.e. there may be a quality component to nutrient reserves that has not been considered so far. In the next chapter I will consider the effects of the quantity and quality of protein reserves.

Chapter 4 - The effect of the quality of a bird's protein reserves on laying.

Introduction

In chapter 3 a significant loss of protein from female pectoral muscle was demonstrated during egg production. The amount of muscle protein lost did not, however, relate to clutch size, mean egg mass or clutch mass which might have been expected if the absolute quantity of protein available in muscle was the determinant of egg laying ability. These trials looked simply at the absolute size of the protein reserves and the quantity used during egg production. The real situation, however, may be more complex. Egg production in zebra finches may be affected by variation in the quality, as well as the size of their protein reserves.

In this chapter I consider the effects of protein reserves of different quality on egg production. Very little is known about whether quality can limit egg production. It is known, however, that egg proteins contain unusually high concentrations of some essential amino acids such as the sulphur amino acids, methionine and cysteine, which are found at far higher concentrations in egg proteins than in plant proteins and many animal proteins (Harvey, 1970). Some of these essential amino acids may not be available in adequate quantities in the diet during the period of egg formation. This causes an imbalance between the requirements of egg formation and the dietary provision of amino acids (Houston, Donnan and Jones, in press *a*). It may be these specific limiting amino acids which could be derived from endogenous protein reserves. A bird with high quality muscle reserves would then be a bird whose muscle proteins contained a high proportion of these limiting amino acids, and which could mobilise them at the time of egg formation.

There is some experimental evidence for the use of specialised protein reserves by birds. Houston, Donnan and Jones (in press *a*) have demonstrated the selective use of

the pectoral muscles as a protein resource during egg production in zebra finches. A loss of muscle sarcoplasm has been attributed to a single, unidentified protein of high molecular mass (Houston, Donnan, Jones, Hamilton and Osborne, in press). As suggested by Kendall, Ward and Bacchus (1976) such a sarcoplasm protein could act as an amino acid reserve without impairing the contractile function of the muscle, and might be able to store particularly those amino acids which are limiting in the diet. There is also evidence that protein quality can limit egg production. In a field study of food supplementation in lesser black-backed gulls Bolton *et al.* (1993) have shown that the quality of protein in the diet during laying can greatly affect egg size. Birds fed a supplementary protein food based on egg proteins produced larger eggs than those fed fish protein. This indicates a mismatch in the amino acid balance of the food supply with the requirements of egg production that can be alleviated by supplementary feeding of protein of specific quality during laying.

This chapter presents experiments to investigate whether protein quality in the diet prior to breeding might influence egg production, presumably through the mediation of protein storage mechanisms. I start with the assumption that the seed diet of zebra finches may be nutritionally inadequate in amino acid balance for egg protein synthesis. Birds fed on this diet may therefore have limited opportunities to build up specific storage protein in their muscles. In the wild this might require a period of sustained favourable conditions. If, however, birds are fed on a diet containing egg proteins, they may have the opportunity to lay down storage proteins that could subsequently be mobilised for egg formation.. One group of females was therefore provided with a diet containing a high proportion of egg proteins and I have called these individuals 'high quality protein' (HQP) birds. A control group were maintained on the basic seed diet and these birds I have called 'low quality protein' (LQP) birds.

Following this dietary treatment the birds were paired and female pectoral muscle masses were estimated from the muscle profiles. From these an index of muscle

condition was produced by correcting for body size. Both groups were then treated identically and given the same low protein diet of seed on which to breed and produce eggs. A knowledge of the size of the protein reserves formed in the two treatments allowed an assessment of the effect of reserve size on egg production, as in chapter three. Between-treatment differences that remained unexplained would therefore have been due to the quality of the protein reserves. The aim of this chapter is therefore to determine the interactions between the diet during the pre-laying period, the size of the resulting protein reserves, and subsequent breeding performance.

Methods

Fifty-five females were put on a basic, maintenance diet of *Panicum* millet for three months. They were then divided between two treatments. Twenty-seven birds continued with this relatively low protein diet for a further two weeks whilst the other twenty-eight were fed mixed seed and a high protein supplement. The supplement was produced by pulverising a large hard-boiled chicken egg with an electric mixer and mixing it with 200g of conditioning food (one chicken egg is equivalent in mass to 72 zebra finch eggs). At the end of the fortnight the muscle condition of each female was estimated before pairing (see chapter two). All male birds were provided with mixed seed and conditioning food throughout this time. After pairing, both treatments were given only the low protein *Panicum* millet diet. The cages that had received the 'high protein' diet were cleaned out to remove all traces of the pre-pairing diet and new composted bark was put in. Each day the food was replenished and the nest boxes checked for eggs.

Freshly laid eggs were removed and replaced with varnished plaster dummy eggs. Fresh egg mass was measured immediately on removal from the nest. The eggs were punctured with a fine needle to prevent explosion in the oven, then baked for one hour at 100°C to harden the contents. They were frozen until dissection could take place. The eggs were dissected to separate the yolk, albumen, and shell with its adherent membranes. Each was dried in an oven at 70°C until constant mass was attained. The yolks are the only part of a zebra finch egg that contain measurable quantities of fat (Houston, Donnan and Jones, in press *a*), therefore lipid levels were only determined for the yolks. This was achieved by Soxhlet extraction using chloroform solvent. The yolks were then redried to constant mass in the oven at 70°C. The dry mass of lipid in each yolk was calculated as the difference between the pre- and post-extraction dry masses.

The protein condition of the laying birds was estimated again when laying ceased. Usually the eggs were laid daily, but sometimes there was a day mid-clutch when no egg was laid. Condition was therefore measured on the second day that no egg was laid, to make sure that the clutch was complete and to avoid disturbance during laying.

Results

Two birds laid eggs on the two days immediately following pairing. The eggs must have been developing before the "pre-laying" measurements were taken. Some changes in body mass and muscle mass may already have occurred, so these birds have been removed from the analysis.

The effect of dietary treatment on pre-laying body reserves

Prior to pairing one group of females had been placed on a diet high in egg proteins for two weeks whilst the other was on a maintenance diet, yet at pairing there was no treatment difference in mean muscle condition (figure 4.1, table 4.1) or mean body condition (figure 4.2) i.e. there was no treatment effect on reserve size prior to laying. I therefore assume that any differences in the breeding performance of the two groups are due to differences in the quality of the protein reserves, not purely the size of those reserves.

To breed or not to breed

All of the HQP females laid eggs and only three LQP birds from twenty-six laid no eggs at all. The proportion of layers to non-layers did not vary significantly between treatments (χ^2=1.52, df=1, P>0.05). There was no difference in the sizes of the reserves of non-breeding LQP birds and those about to lay, in fact, mean muscle condition and body condition were slightly higher for non-laying LQP birds than layers (table 4.2), therefore there was no indication of any effect of body reserves on the decision to breed.

The timing of laying

There was no significant difference in the number of days between pairing and laying for HQP birds (mean=9.5 days, SE=1.0, n=27) and LQP birds (mean=11.3 days, SE=1.6, n=23) (t_{38}=-0.94, P=0.351). When the two treatments were pooled the timing of laying did not correlate with the body condition of females at pairing (r_{50}=-0.170, P=0.238), body mass (r_{50}=-0.155, P=0.284), muscle mass (r_{50}=-0.085, P=0.556) or muscle condition (r_{50}=-0.100, P=0.491).

Egg production

Treatment differences in egg production - the effects of reserve quality.

HQP birds laid significantly larger clutches, heavier eggs and greater total clutch masses than LQP birds (figures 4.3 to 4.5 and appendix 1). These are highly significant differences, and yet the diet during laying was the same for both treatments. An analysis of the proportions of the major egg components with egg mass and by female dietary treatment is detailed later.

A relationship between clutch size and the number of days between pairing and laying was expected, as in chapter 3, however, only the data for LQP birds showed this (figure 4.6). This may be because HQP birds all appeared to lay large clutches and began laying soon after pairing. LQP birds laid smaller clutches, and the smallest clutches, of only one or two eggs, were laid later than larger clutches.

Treatment differences in post-laying body reserves and the use of reserves

Females in both treatments lost large amounts of estimated muscle mass over the laying period (HQP, t_{24}=5.19, P<0.001; LQP birds, t_{19}=7.25, P<0.001). The mean

change in muscle mass was greatest for LQP birds (table 4.1, figure 4.7) even though the egg production of HQP birds was much greater (figures 4.3 to 4.5).

There were no differences in muscle mass or muscle condition between the two treatments at the cessation of laying (table 4.1, figure 4.1), but there were differences in body mass and body condition (table 4.1, figure 4.2). LQP birds completed laying in significantly lower body condition than HQP birds. The mean body mass for HQP birds rose during the laying period (paired t-test: HQP birds t_{24}=-2.12, 2-tailed P=0.044) whilst the mean for LQP birds appeared to fall a little, but not to a significant degree (LQP t_{19}=1.59, P=0.129). These changes in body mass was significantly different between treatments (table 4.1).

Within treatment variation - the effects of reserve size on clutch size, mean egg mass and clutch mass

A full list of results of correlations between the levels of body reserves and clutch sizes, mean egg masses and clutch masses is given in appendix 2. Only correlations of significance are detailed here. For HQP birds the degree of muscle loss was correlated with the number of eggs laid (figure 4.8): the birds which laid the largest clutches, lost the most muscle mass. LQP females lost more protein than HQP birds laying the same clutch sizes, but the degree of loss did not correlate with clutch size. Thus the relationship between clutch size and the use of body proteins may not be affected solely by the size of a bird's protein reserve before laying, but possibly also by the quality of that protein. HQP birds, therefore, lost muscle mass in a quantity proportional to the clutch size produced, whereas LQP birds lost a large amount of protein regardless of the number of eggs laid. The result was that although HQP birds laid more eggs, and larger eggs, than LQP birds, the LQP birds lost more pectoral muscle lean dry mass (figure 4.7). The effect was even more pronounced when the loss per gram of egg laid was considered (figure 4.9, table 4.1).

Egg production and the size of protein reserves

In neither treatment did pre-laying or post-laying muscle condition relate to clutch size, mean egg mass, clutch mass, or the number of days between pairing and laying.

The pre-laying, the post-laying, and the change in body condition over the period of egg production were tested against clutch size, mean egg mass and clutch mass by correlation analysis. The data for each treatment were tested separately. For LQP birds, the change in body mass over the period of egg formation was correlated with mean egg mass (figure 4.10). A negative relationship might have been expected; birds making the greatest investment in their eggs, losing body reserves, and laying the largest eggs. However, there was a positive relationship, i.e. heavy eggs were laid by females that gained body condition and light eggs by females that lost body condition. This could be because the birds that laid eggs of greater than 1g were the few birds in this treatment that also were in a position to increase their body masses, whereas the others lost mass to varying degrees but could only lay light eggs.

Clutch size was correlated with both body mass and body condition following laying, but only for LQP birds (figures 4.11 and 4.12). There was no relationship between the change in body condition and clutch size for either HQP or LQP birds (figure 4.13). These results back up the results of chapter 3, in which birds were found to complete laying with body masses in proportion to the number of eggs that were laid. Here, for LQP birds body mass gave a stronger relationship with clutch size than body condition, indicating either that the size of a bird may not affect the size of the fat stores required after laying, or that the method of determining condition does not produce a good index and may add unexplained variation to the relationship. HQP birds completed laying with greater body condition (figure 4.2). For these bird, body mass may not have been a factor in the decision to stop laying because they may have had more fat than required for an insurance against weight loss during the incubation

period. There were no correlations between clutch size and the change in body mass during laying (figure 4.13), which more often rose than fell in the HQP birds. Body mass at the cessation of laying is therefore not the result of energy stress during egg formation and it is not likely that the essential fatty acids required by the eggs were limiting egg production, unless these are not stored within the regular fat reserves.

Egg analysis

To test the effects of the treatments on the levels of egg components the effect of variation in fresh egg mass must be taken into account. However, because the egg components are constituents of egg mass, the mass of egg water was used to make allowance for variation in egg mass. Egg mass and the mass of egg water are very highly correlated in both treatments (HQP r_{93}=0.991, P<0.001; LQP r_{50}=0.995, P<0.001). Covariance analysis was used. Clutch means were analysed because the pre-laying dietary treatment will directly affect the laying females, not the eggs. Each egg represents a sample of the effect of a treatment on a particular female. The null hypothesis was that the pre-laying dietary treatment of the laying female did not affect the dry mass of the egg components after allowing for differences in the mass of egg water. The only egg component that showed a treatment effect was shell dry mass (table 4.3). The gradients for the two groups of birds were similar but the elevation of the regression lines for clutches from LQP birds was significantly lower than for HQP birds (figure 4.14). The other relationships are graphed in appendix 3.

The proportions of these egg components were compared using clutch means. Only the egg contents were considered. This removes the effect of the treatment on shell dry mass. The two tables show that the egg contents remained in proportion regardless of the mass of egg water (table 4.4).

Table 4.1 - A comparison of the sizes of body reserves and muscle reserves for females that received a diet of either "high quality protein" (HQP) or "low quality protein" (LQP) prior to breeding. The table shows the sizes of reserves before and after egg formation and the change in their size during this time.

Variable	Diet	Mean	SE	n	t-test
Pre-lay body mass (g)	HQP	14.155	0.186	27	t_{51}=0.35, P=0.726
	LQP	14.066	0.166	26	
Pre-lay body condition	HQP	0.022	0.178	27	t_{51} = 0.19, P=0.853
	LQP	-0.023	0.156	26	
Pre-lay muscle mass (g)	HQP	0.764	0.019	27	t_{51}=-0.19, P=0.848
	LQP	0.769	0.016	26	
Pre-lay muscle condition	HQP	-0.005	0.017	27	t_{51} = -0.44, P=0.661
	LQP	0.005	0.016	26	
Post-lay body mass (g)	HQP	14.583	0.269	25	**t_{35}=2.65, P=0.012**
	LQP	13.784	0.137	20	
Post-lay body condition	HQP	0.459	0.253	25	**t_{36}=2.83, P=0.008**
	LQP	-0.349	0.133	20	
Post-lay muscle mass (g)	HQP	0.714	0.018	25	t_{43}=0.53, P=0.597
	LQP	0.701	0.016	20	
Post-lay muscle condition	HQP	-0.054	0.016	25	t_{43}=0.63, P=0.530
	LQP	-0.069	0.015	20	
Change in muscle mass(g)	HQP	-0.054	0.010	25	**t_{43}=2.06, P=0.045**
	LQP	-0.086	0.012	20	
Change in muscle mass	HQP	-0.010	0.002	25	**t_{29}=4.12, P<0.001**
per gram of egg laid	LQP	-0.029	0.004	20	
Change in body mass(g)	HQP	0.450	0.212	25	**t_{43}=2.58, P=0.014**
	LQP	-0.306	0.193	20	

Table 4.2 - Comparison of body reserve sizes for non-breeding LQP females and for those about to lay.

	Non-breeders (n=3) Mean, SE	Breeders (n=23) Mean, SE	t-test
Body mass (g)	14.215, 0.459	14.047, 0.181	t_{24}=0.32, P=0.753
Body condition	0.355, 0.459	-0.072, 0.167	t_{24}=0.87, P=0.395
Muscle mass (g)	0.748, 0.019	0.772, 0.086	t_{24}=-0.48, P=0.638
Muscle condition	0.011, 0.019	0.004, 0.018	t_{24}=0.14, P=0.890

Table 4.3 - The results of analyses of covariance to test for differences between the major components of eggs laid by HQP and LQP females, after allowing for variation in the mass of egg water. The mean data for each clutch were used.

Egg constituent	Difference in gradients	Difference in elevations
Shell dry mass	$F_{1,32}$=0.29, P=0.594	**$F_{1,33}$=56.23, P<0.001**
Albumen dry mass	$F_{1,32}$=0.36, P=0.550	$F_{1,33}$ = 0.09, P=0.761
Yolk fat dry mass	$F_{1,32}$=0.00, P=0.966	$F_{1,33}$ = 0.06, P=0.808
Yolk lean, dry mass	$F_{1,32}$=0.77, P=0.386	$F_{1,33}$ = 0.15, P=0.700

Table 4.4 - Spearman's rank correlation coefficients for the proportions of the major egg components (excluding the shell) with fresh egg mass, for females with high quality protein (HQP) or low quality protein (LQP) reserves. The mean data for each clutch were used.

Egg component	HQP	LQP
% water	r_{22}= 0.011, P=0.960	r_{16}= 0.250, P=0.350
% albumen dry mass	r_{22}=-0.122, P=0.589	r_{16}=-0.361, P=0.170
% yolk fat dry mass	r_{22}=-0.011, P=0.962	r_{16}= 0.250, P=0.350
% yolk lean, dry mass	r_{22}=-0.096, P=0.673	r_{16}=-0.396, P=0.129

Figure 4.1. Change in the muscle condition of HQP and LQP females during the period of egg formation. The standard errors are shown.

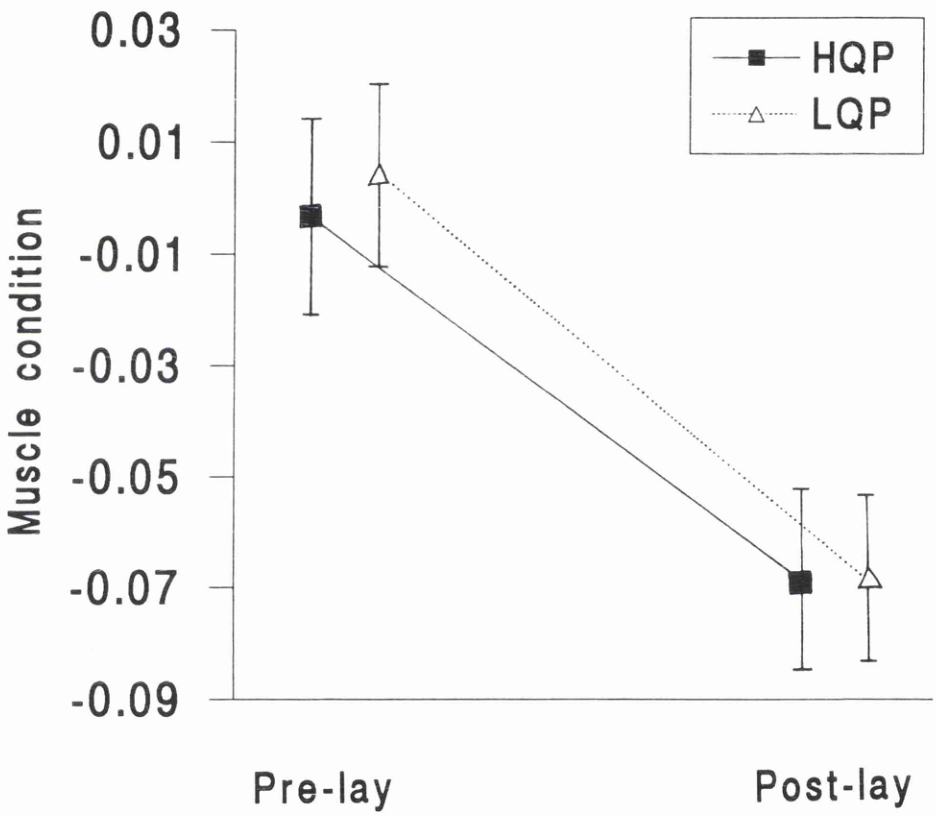


Figure 4.2. **Change in the body condition of HQP and LQP females during egg formation. The standard errors are shown.**

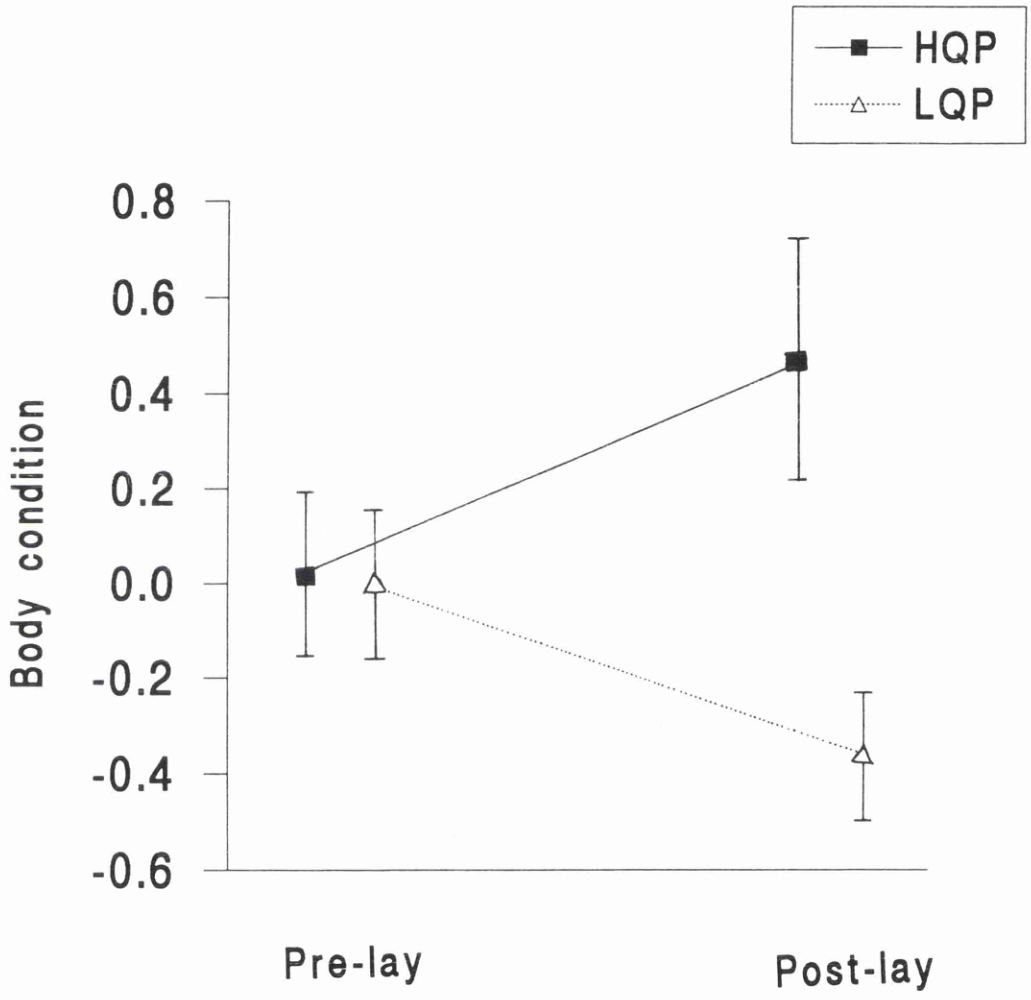


Figure 4.3 Comparison of the median clutch sizes laid by HQP (n=27) and LQP (n=26) females with inter-quartile range bars. Mann-Whitney U test: U=175, Z=-3.19, P=0.001.

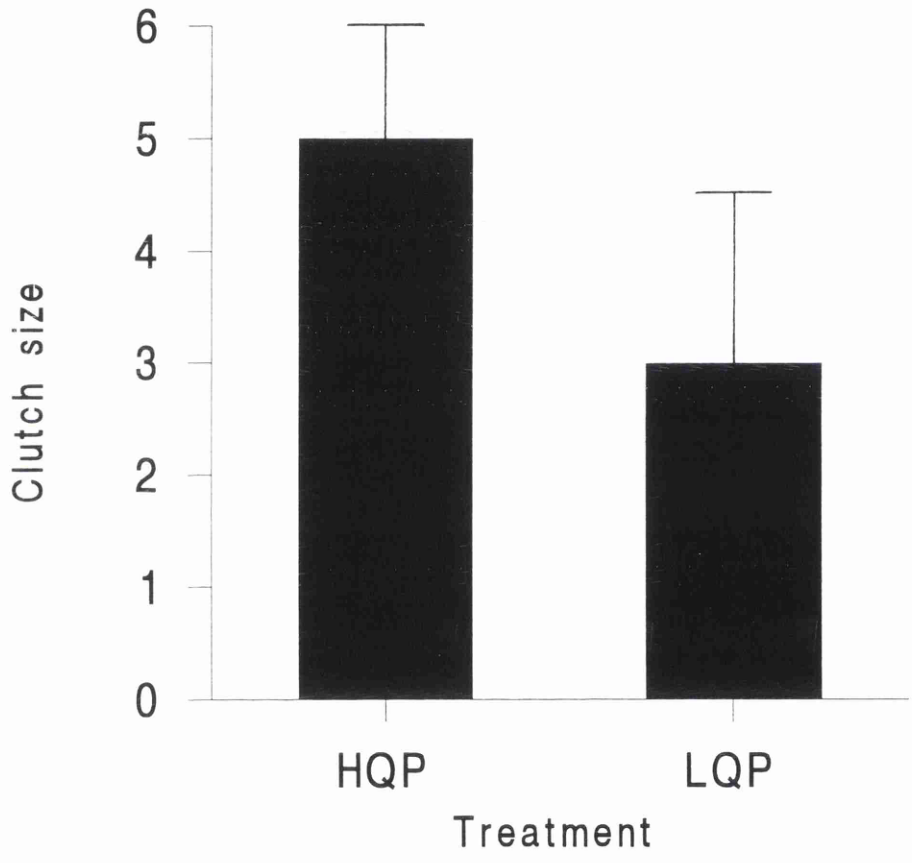


Figure 4.4 Comparison of the mean egg masses for each clutch, laid by HQP (n=26) and LQP (n=23) females; t-test t=3.83, P<0.001, df=47. Standard error bars are given.

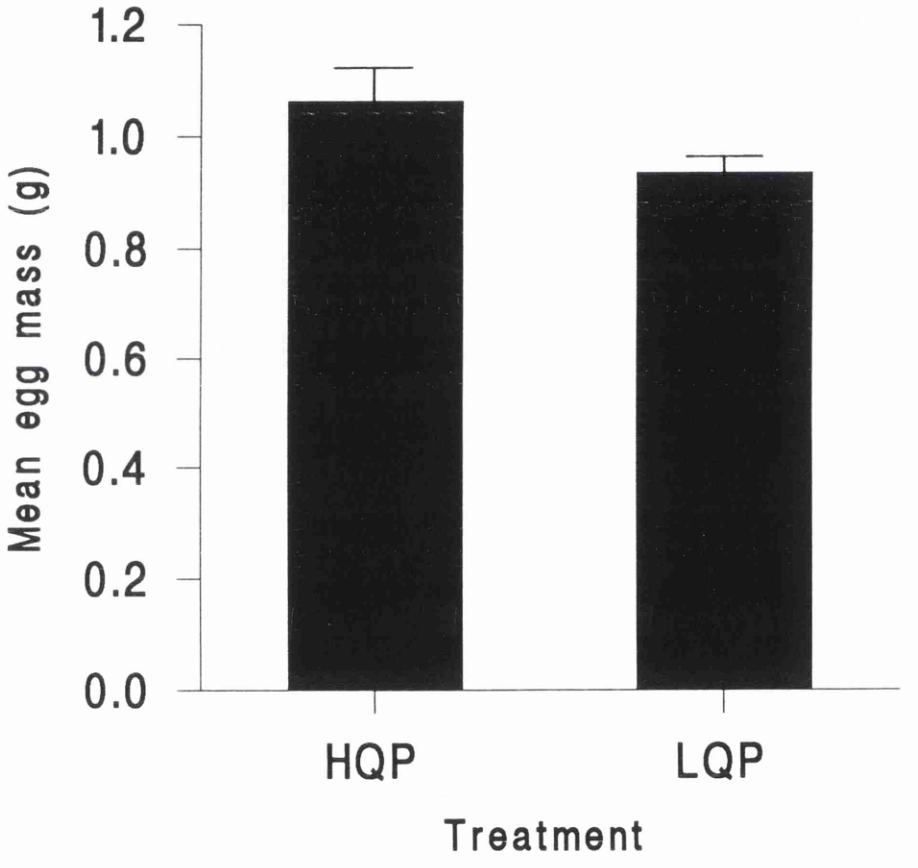


Figure 4.5 Comparison of the clutch masses laid by HQP (n=26) and LQP (n=19) females; t-test t=4.47, P<0.001, df=50. Standard error bars are given.

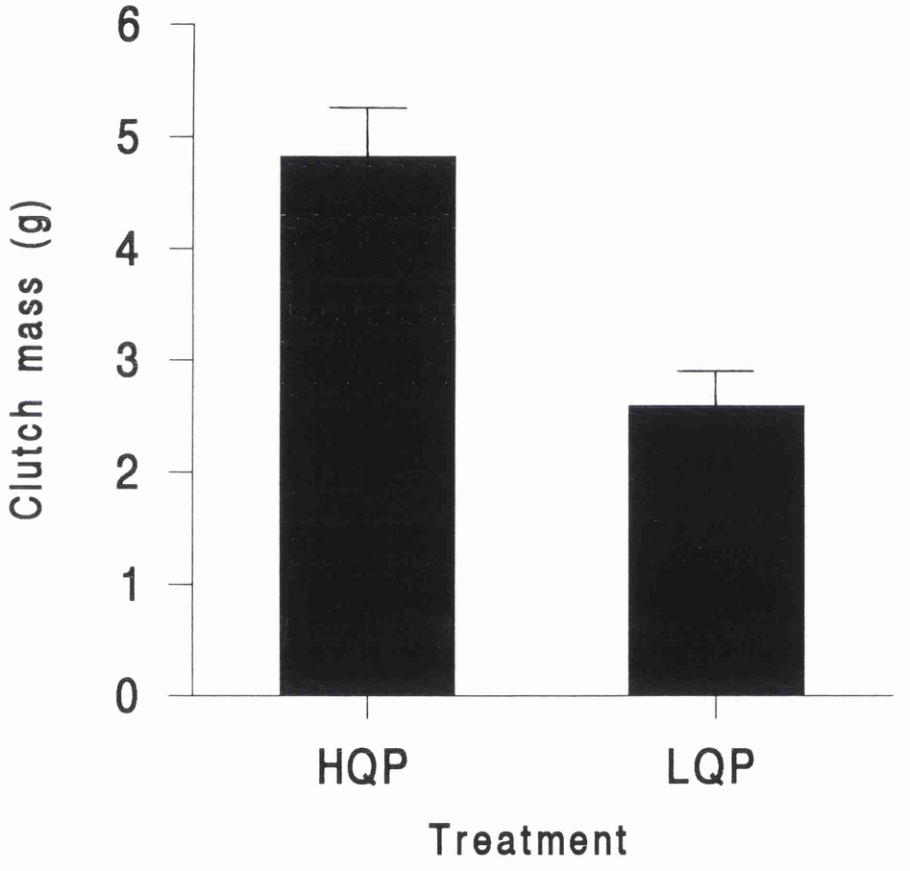


Figure 4.6. Correlations between clutch size and the number of days between pairing and laying for HQP and LQP birds.
HQP: r=-0.001, P=0.997, n=27
LQP: r=-0.518, P=0.011, n=23

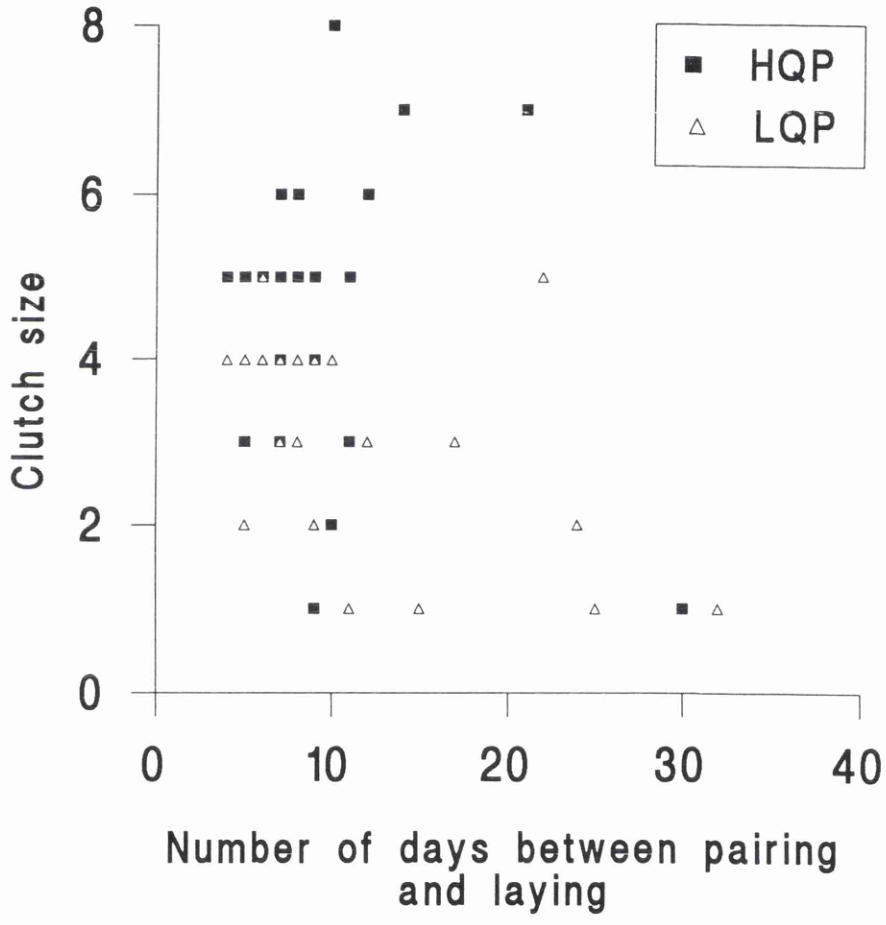


Figure 4.7 The loss of lean, dry mass from the pectoral muscles of HQP and LQP females (t-test, t=4.12, df=29, P<0.001). Standard error bars are given.

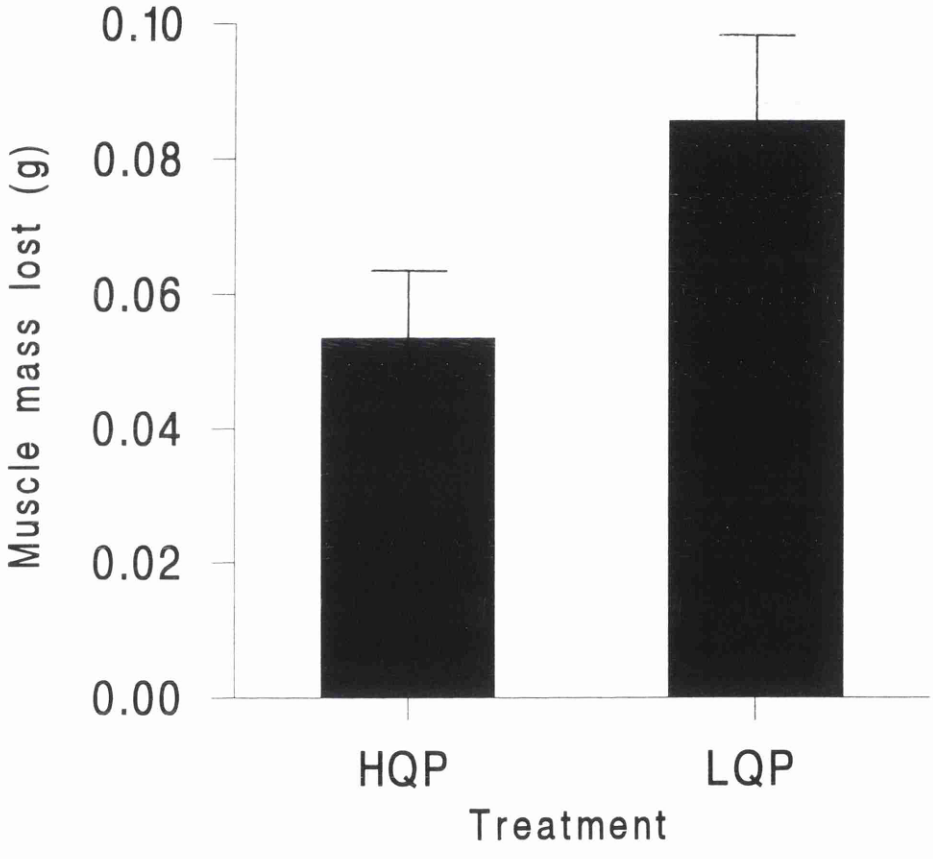


Figure 4.8. **Correlations between clutch size and the change in muscle mass of HQP and LQP females.**
HQP: Spearman's r=-0.485, P=0.014, n=25
LQP: Spearman's r=-0.083, P=0.714, n=20

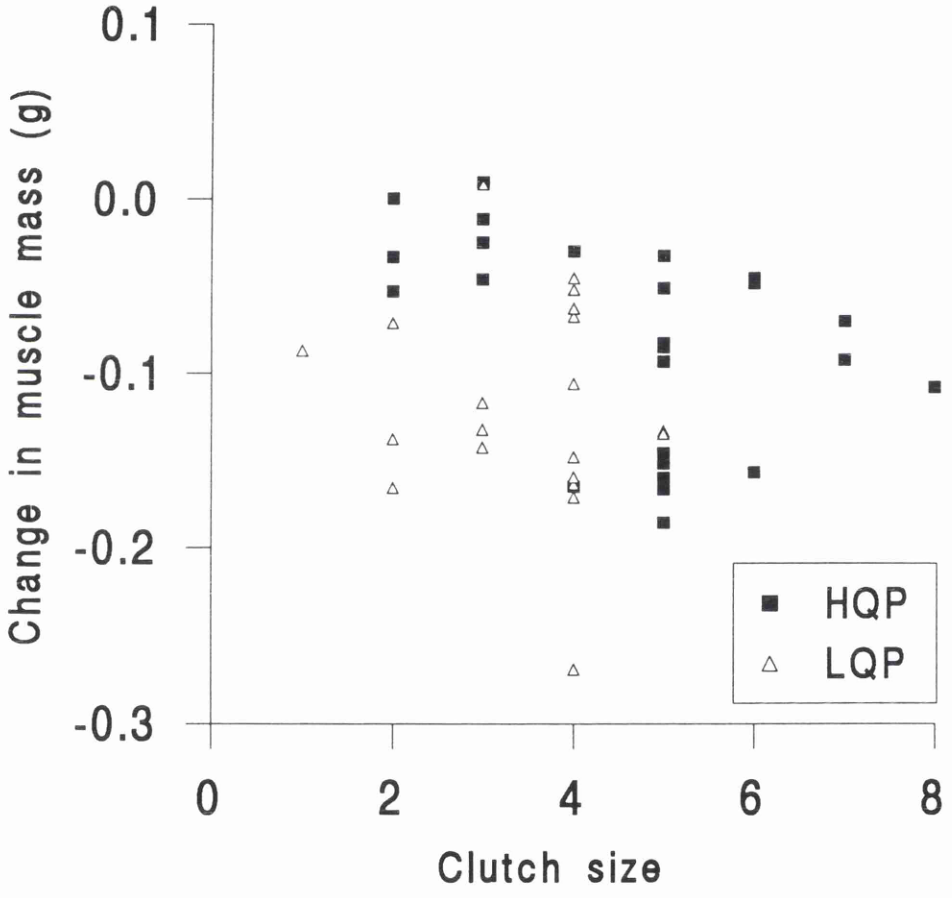


Figure 4.9 The loss of lean, dry mass from the pectoral muscles, per gram of egg laid, for HQP and LQP females (t-test, t=4.12, df=29, P<0.001). Standard error bars are given.

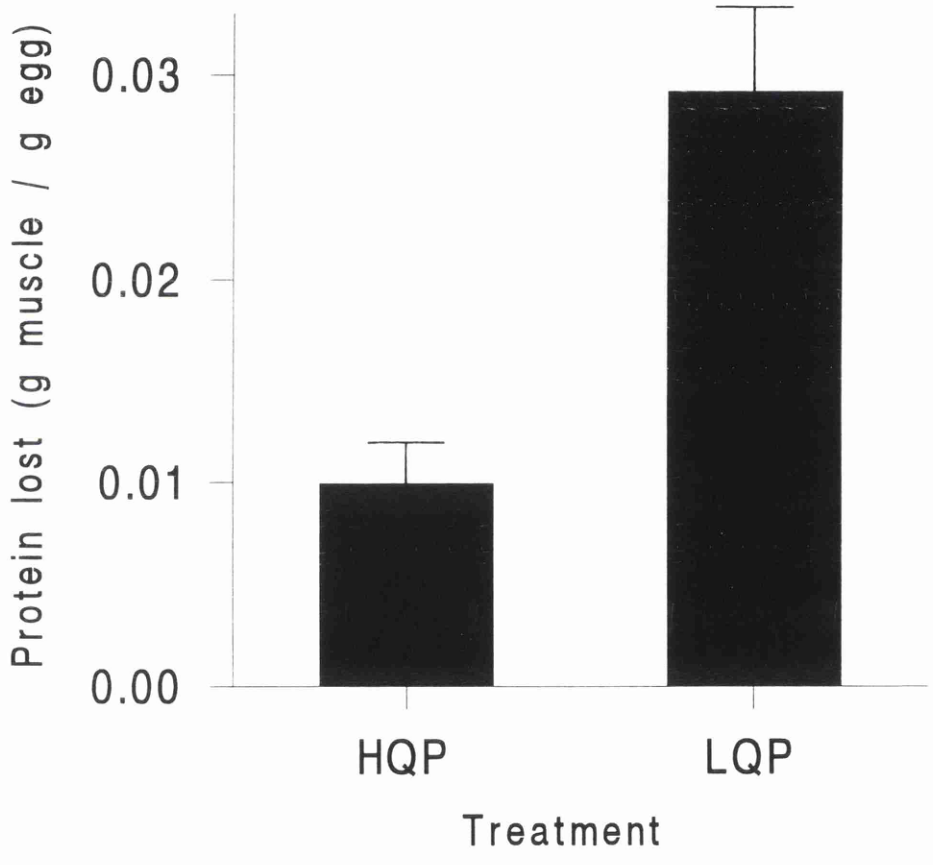


Figure 4.10. The change in female body mass plotted against mean egg mass for LQP and HQP females.

HQP: Pearson's r=0.263, P=0.204, n=25

LQP: Pearson's r=0.555, P=0.011, n=20

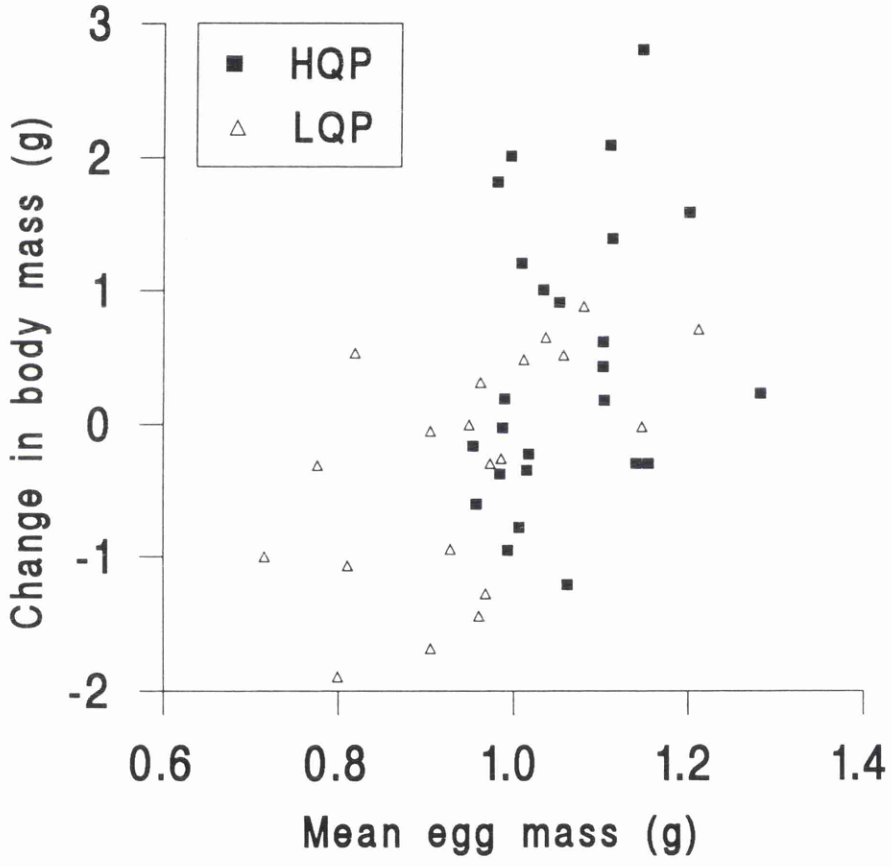


Figure 4.11. Correlations of post-laying body mass with clutch size for HQP and LQP birds.
HQP: Spearman's r=-0.390, P=0.054, n=25
LQP: Spearman's r=-0.503, P=0.024, n=20

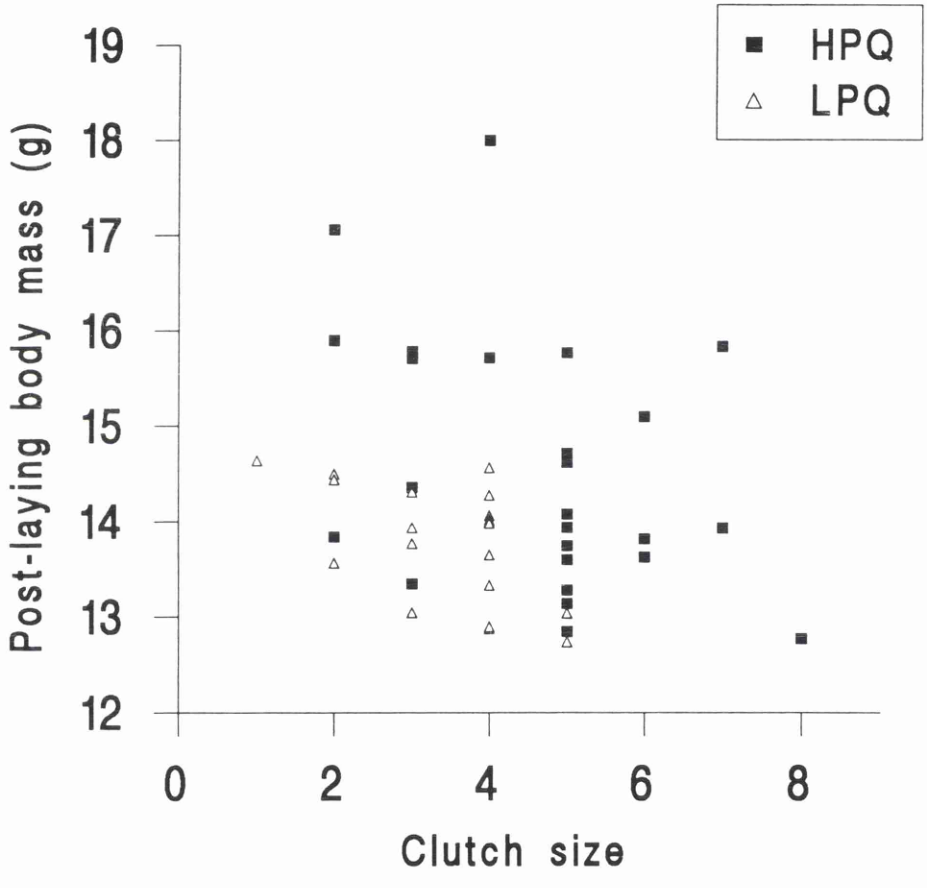


Figure 4.12. Correlations between post-laying body condition and clutch size for HQP and LQP birds.
HQP: Spearman's r=-0.359, P=0.078, n=25
LQP: Spearman's r=-0.451, P=0.046, n=20

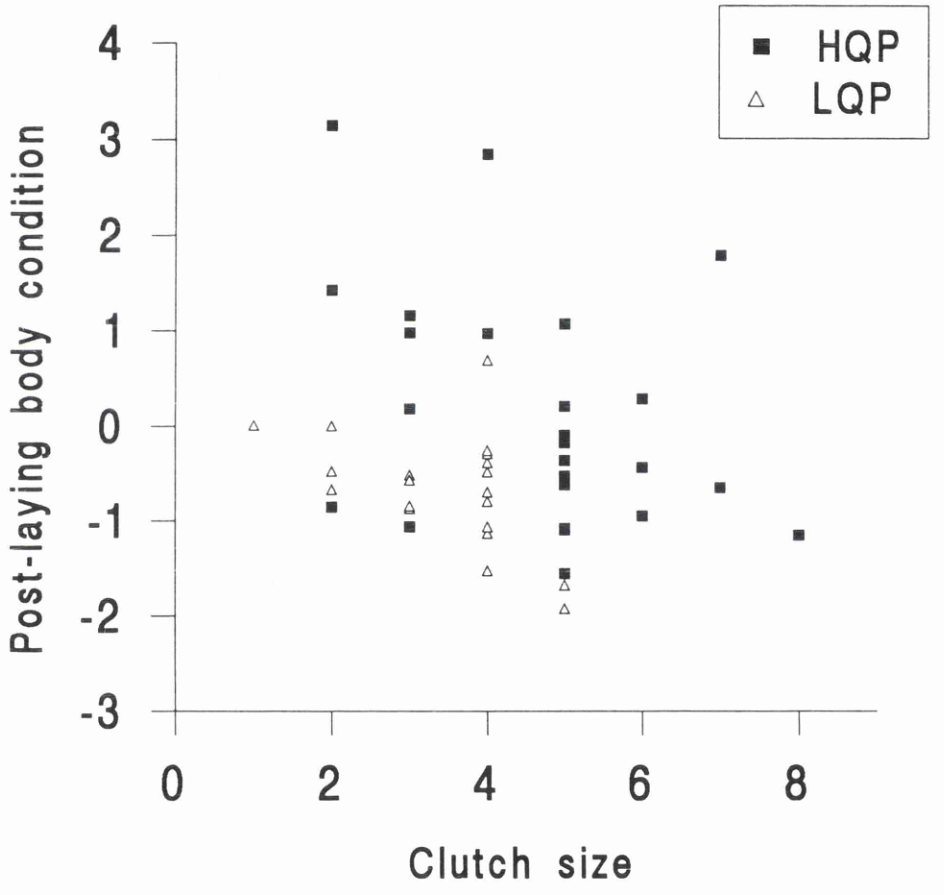


Figure 4.13. Correlations between clutch size and the change in body mass of HQP and LQP females.
HQP: Spearman's r=-0.178, P=0.395, n=25
LQP: Spearman's r=-0.352, P=0.128, n=20

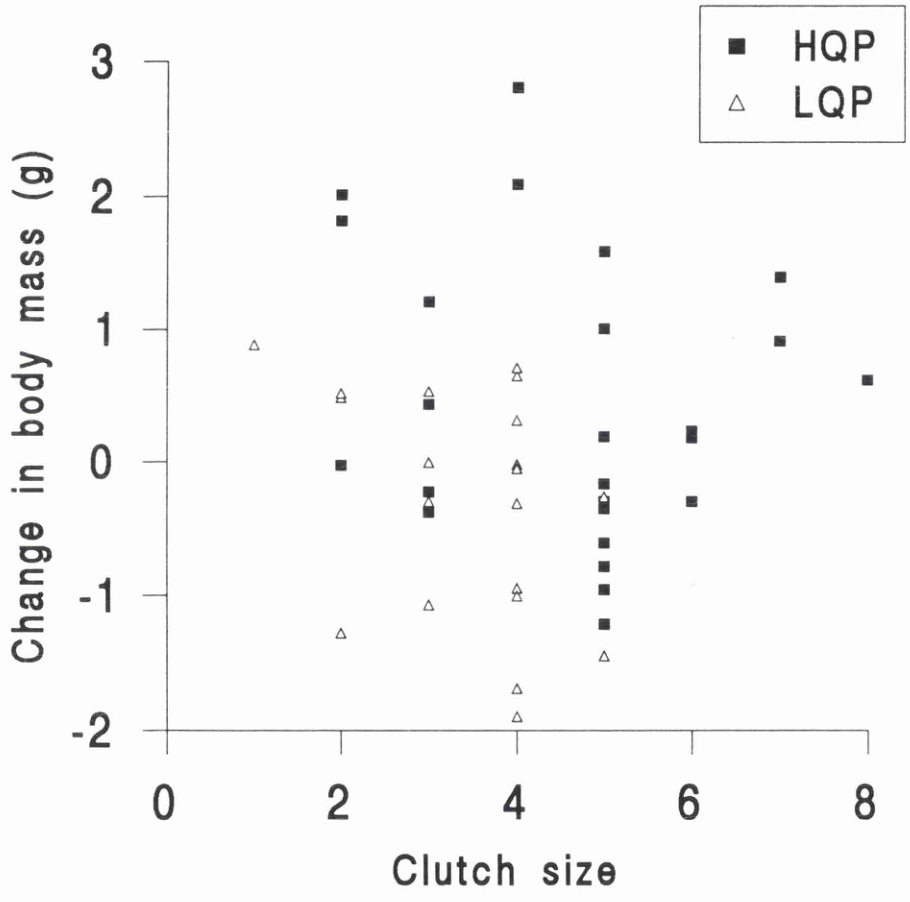
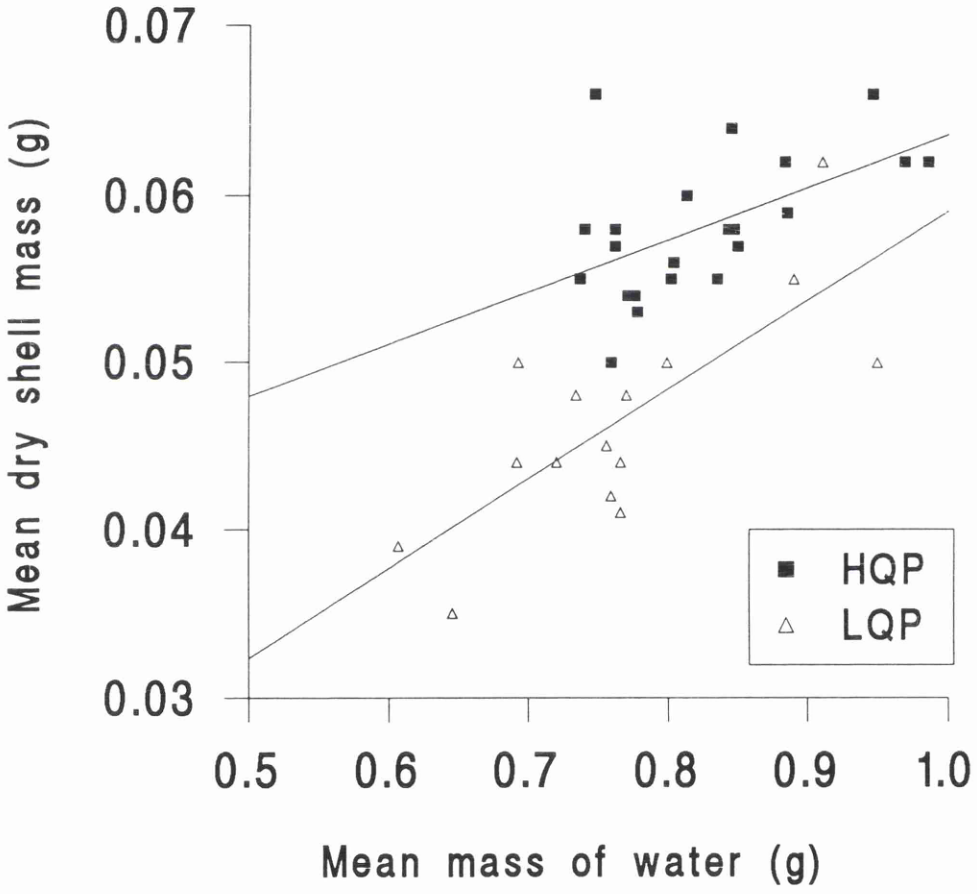


Figure 4.14. Comparison of the mean shell dry masses of eggs from HQP and LQP females. Mean clutch data were used. Gradients $F_{1,32}=0.29$, $P=0.594$; elevations $F_{1,33}=56.23$, $P<0.001$. The regression lines are shown for comparison.

HQP: $y = 0.032 + 0.031x$

LQP: $y = 0.006 + 0.054x$



Discussion.

Egg production and reserves of fat and protein

HQP females laid, on average, one egg more than LQP birds and their eggs were heavier. The difference was astonishing since the only difference between the two treatments was the diet of the females prior to pairing. The treatment differences in egg production are therefore presumably due to the effects of differences in the reserves of females. Bolton *et al.* (1993) suggested that in lesser black-backed gulls the number of follicles ovulated, and therefore the clutch size, was determined by the levels of flight muscle protein, but egg size (particularly albumen size) was influenced more by dietary intake during egg formation than the levels of body reserves. Houston, Donnan and Jones (in press *b*), however, have shown that reserve proteins are moved into both the yolk and the albumen during egg formation in zebra finches and might therefore be expected to influence egg mass. My results show that both clutch size and egg mass can be affected by the reserves laid down prior to breeding. The pre-pairing diet did not, however, affect the sizes of the reserves: both the body condition and the protein condition of females prior to egg production was similar in each of the treatments and the diet during laying was the same for each treatment, so the observed differences in egg production must have been caused by variation in the quality of reserves, since LQP birds lost no less protein than the much more productive HQP birds.

Females in both treatments lost protein from their pectoral muscles during egg production, but HQP birds lost it in proportion to the clutch size laid. This could have been because the highly productive group had the opportunity to build up selectively a large store of the amino acids that might otherwise limit a female's potential for egg production. The LQP females on average lost more protein than HQP even though the HQP birds laid larger clutches and larger eggs. It is unlikely that this was all used

in the provisioning of the eggs because relatively small eggs and small clutches were laid. It is possible that a lot of protein was deaminated. If only small quantities of the amino acids in shortest supply during egg formation were stored before pairing, then muscular protein may have been lost in large amounts in an attempt to obtain just small amounts of these amino acids. A loss of both the sarcoplasmic and the myofibrillar content of the pectoral muscles has been demonstrated by Houston, Donnan, Jones, Hamilton and Osborne (in press) in the zebra finch. Sarcoplasm loss was due to a reduction in an unidentified protein of high molecular weight which may form a specific protein reserve. It could be that the HQP birds used such a reserve during egg formation, reducing the volume of their pectoral muscles to an extent proportional to the number of eggs laid. The LQP birds had muscles of a similar range in size but they may have had very low levels of the proposed reserve protein. The production of eggs would then have drained the pool of amino acids which is also used constantly to reform body proteins, which are broken down at quite a constant rate (Millward, Garlick, Nnanyelugo and Waterlow, 1976). The myofibrillar content of the muscles would therefore have been reduced, decreasing the muscle volume, but perhaps not resulting in the release of the specific amino acids required in egg formation. This provides a possible explanation for the greater loss of protein from LQP than HQP birds yet the lower clutch sizes and egg masses produced.

Body condition, however, increased in the HQP group; this is an index of protein and fat reserves together. Many field studies have used a similar index to determine the extent to which body reserves are used during egg formation. Hornfeldt and Eklund (1990) suggested that body reserves were not a main source of nutrients for egg formation when their Tengmalm's owls increased in mass at this time, yet the zebra finch may derive 95% of the proteins for a clutch of eggs from body reserves (Houston, Donnan, and Jones, in press *a*). Here I found that they gained mass whilst their protein reserves were reduced. Overall, body mass can therefore mask significant changes occurring in some tissues. Conclusions from a study of body mass must

therefore be very limited, or at least, tentative. It would be much better, wherever possible, to make measurements more specific to separate the effect of fat and protein. Clutch size is therefore related to protein during egg production, but not to the amount of fat. LQP females all lost relatively large amounts of protein (equivalent to the greatest losses of HQP birds), and fat, and yet laid fewer, and lighter, eggs than HQP birds. The quantity of protein used is therefore only relevant to the resulting clutch size when the protein reserve is of specific quality. The HQP females must have sequestered a reserve of proteins useful in egg production, whereas the LQP females built up a reserve of lesser quality. The HQP females, when forming the eggs, then used their protein reserves in proportion with the requirements of the clutch. These reserve proteins may not have come exclusively from the pectoral muscles, but probably also from other parts of the body, such as the leg muscles and gut (Houston, Donnan and Jones, in press, *a*). However the LQP birds, in an attempt to produce a clutch of eggs, appeared to break down large amounts of protein in order to extract a small amount of the proteins required in egg production. Large quantities of pectoral protein were therefore lost, but only relatively small clutches and small eggs were produced compared with the HQP birds.

These results can be related to the models of Jones and Ward (1976) for the role of protein in the control of egg formation (see chapter 2, introduction). Following a further study of the red-billed quelea, Jones and Ward (1979) suggested that this granivorous species relies on fat reserves whilst laying in order to concentrate in foraging for a diet high in proteins (insects) with which to provision the eggs. High quality birds may be most successful, catching the greatest number of insects, so body protein reserves could be used at a lower rate than by less successful hunters. Protein reserves appeared to be used to a similar extent, regardless of the number of eggs forming, but a high protein diet could allow birds to use their protein reserves at a low rate, and so lay for a long period before the reserves became low, producing a large clutch.

In contrast, zebra finches appear to rely heavily on their protein reserves (Houston, Donnan and Jones, in press *a*), as a result of their specialisation for breeding while on a low protein diet (Morton and Davies, 1983; Zann and Straw, 1984). This chapter has demonstrated that the extent of protein reserve use is related to the number of eggs laid when birds have the opportunity to build up a reserve of high quality. This fits the predictions of models (a) and (b) of Jones and Ward (1976), but there was no relationship between the condition of birds before, or after, laying and the number of eggs laid. It therefore seems more likely that the level of protein reserve use is a product of the number of eggs laid, not the other way around, i.e. in the zebra finch clutch size is not controlled by the size of a bird's protein reserves (or fat reserves) but the quality of body reserves can influence clutch and egg size. The dietary protein intake of a bird may not be great enough, or of high enough quality, to allow a reduction in the rate of reserve use and so a lengthening of the laying period that could result in a greater clutch size. Instead, the dietary intake may be reduced (chapter 3) and provides mostly carbohydrate as an energy resource, whilst egg proteins may be formed mainly from body reserves. These may be specialised for the requirements of egg formation (Houston, Donnan, Jones, Hamilton and Osborne, in press).

Laying behaviour

Davies (1977) has suggested that body condition could have an effect on whether a zebra finch can breed or not. He observed that in a poor year for breeding, body masses had been lower in the preceding winter. The data here suggest that the birds will mostly breed irrespective of their body condition or protein condition, although these captive birds were not in conditions causing great nutritional stress, as might sometimes occur in the arid interior of Australia that this species inhabits. The three non-breeders did not appear to be limited in the size of their reserves. The high

proportion of LQP birds that bred indicates that reserve quality did not exert a limiting effect on the decision to breed. It is more likely that the three non-breeders were in infertile pairs.

Laying date, although related to female body condition before breeding in chapter 3, shows no such relationship in this data. Whilst the sample size was much smaller here, if this relationship is not demonstrable in a sample of 50 birds then it is probably quite a weak relationship. Reserves of fat may turn out to be important in the decision to lay, but this was not so in this sample. In the wild, where birds could be more energy stressed, this may important. No treatment effect was shown, so there was no effect of reserve quality.

Egg analysis

A treatment difference in egg mass has been demonstrated. This may be due to variation in overall egg mass, the egg components remaining in proportion, or to variation in the relative masses of individual egg components. Here the egg components remained in the same proportions regardless of egg mass. Body reserves, apparently were used in the provisioning of both the yolk and the albumen. This supports the results of Houston, Donnan and Jones (in press *b*) who showed that body proteins were used in the production of both yolk and albumen in all the eggs of a clutch. There was a treatment difference in egg shell dry mass after allowing for variation in egg water (which is very closely related to egg mass) by analysis of covariance: LQP birds laid eggs with lower shell masses than those of the same size from HQP birds.

Shell thickness can be affected by many factors: a lack of calcium, ascorbic acid, linoleic acid, or vitamin D, a change in the electrolyte balance of the blood (altering its bicarbonate content), or an excess of phosphorus or manganese (Scott, Nesheim and

Young, 1982). The causative factor here has not been identified, but calcium limitation would seem unlikely because cuttle bone and oyster shell grit were provided *ad libitum.* This could have occurred if the birds chose to maximise their seed intake at the expense of their calcium intake, leaving a calcium deficit during shell formation. However, in chapter three, breeding pairs were shown to have a lower seed intake than non-breeding pairs. Seed intake during egg formation correlated negatively with clutch size, but not egg mass, although the daily requirement for calcium should have been related to egg size, not clutch size. This indicates that the reduction in seed intake is not related to a requirement for calcium, but most likely linked to either the release of body reserves or a reduction in the time spent feeding overall. It is therefore unlikely that there was a calcium deficit due to a strong feeding preference for seed, to the exclusion of the calcium required in the diet during laying. Calcium was provided *ad libitum* to all birds in the form of cuttle bone. Pre-laying diet therefore affects either the behaviour of a female in collecting calcium for egg shell production, or the ability of a female to absorb calcium in the gut or utilise it in the shell gland. It is possible that either the uptake of calcium from the digestive tract, the quantity dissolved as bicarbonate in the blood, or the ability of the shell gland to form good egg shell might be impaired by some other nutritional factor.

In summary, the size of high quality protein reserves affected the number of eggs laid, and the quality of protein or other reserves affected the mass of the eggs produced. Therefore a bird with a reserve of high quality protein can make use of this to lay a large clutch size and large eggs, whereas a bird with only a low quality protein reserve, possibly lacking in certain amino acids, may mobilise large amounts of protein, but still have only a low capacity for egg production, both in terms of egg size and number. However there was no information regarding the effects of these differences on breeding success. The next step was therefore to repeat this experiment, allowing the eggs to hatch, and determine whether differences in egg

mass and egg quality can affect the number of birds hatching and their growth to fledging.

Chapter 5 - The residual effects of pre-laying diet on the viability of the eggs subsequently laid, and the growth of the chicks produced.

Introduction

In chapter 4 egg mass was related to the quality of the reserves of laying females. Egg mass might affect the likelihood of an egg hatching and a chick's growth and survival to fledging, but such ideas have received little attention (Perrins and McCleery, 1994; Williams, 1994). This chapter addresses this hypothesis, using the zebra finch as a model, and also tests whether there are also egg quality effects, i.e. biochemical or structural differences that do not affect egg mass since the proportions of the major egg components do not vary with egg mass or the pre-laying dietary treatment of the female parent (chapter 4), produced by differences in the reserves of the laying birds. Effects might be seen at the egg stage, as variation in hatchability, or hatchling size or condition, or at the chick stage, as differences in the rates of growth, or fledging size or condition. The body reserves of laying birds might also affect their incubation behaviour. There are therefore three ways in which parental body reserves might affect the viability of the offspring they produce, by affecting the masses of the eggs laid, the quality of those eggs, or the behaviour of the parents during incubation.

Egg mass has been found to affect both the viability of eggs (Perrins and McCleery, 1994), and the growth of the resulting chicks (Magrath, 1992; Schifferli, 1973), but the evidence is both equivocal and very sparse for passerine species (Williams, 1994). For instance, no effects of egg mass on offspring growth were demonstrated by Ojanen (1983) in the pied flycatcher, *Ficedula hypoleuca*, or Bancroft (1984) in the boat-tailed grackle, *Quiscalus major*. I have found no studies of the effects of egg mass in the zebra finch. The quality of the pre-laying diet of the female, however, can have a strong effect on the masses of the eggs subsequently produced (chapter 4). If there is a significant effect of egg mass on egg viability or offspring growth, then there

could be a profound effect of maternal pre-laying diet on the likelihood of each egg hatching, and the resulting chick fledging. This will be addressed in the zebra finch to determine whether this species follows the general theory that in birds egg mass affects chick condition more than chick size (Williams, 1994).

Two experiments were designed to determine firstly whether hatching success is affected by egg mass, egg quality, or parental behaviour, secondly, whether egg mass or egg quality affect chick size or condition at hatching, and finally, whether chick growth rates increase or overcome these differences by the time of fledging.

Methods

Males and females were segregated in partitioned breeding cages, but could hear each other through the screen. The males were all provided with a 'high protein' diet of conditioning food mixed with pulverised, hard-boiled egg and mixed seeds; the females were divided into two groups:

- one group was fed the 'high protein' diet;
- the other group was fed a 'low protein' diet of *Panicum* millet.

After 4 weeks, the partitions were removed and some choice in pairing was allowed to the birds. The partitions were replaced and nest boxes were provided with nesting material. Pairs in both treatments were provided with the same diet of *Panicum* millet on which to produce the eggs and incubate them. Any cages in which the diet was changed at this point were cleaned out to remove all traces of the original diet.

First experimental trial

Fifty-four pairs of birds were used in this trial. The boxes were checked twice a day, firstly for newly laid eggs, which were weighed in grams, to 3 decimal places, and numbered in pencil, and secondly to determine the date of latching for each egg, and measure and weigh the chick before its first feed. The chicks were weighed in grams, to 3 decimal places and three size measurements were recorded, to 0.1mm: head and bill length, wing length, and the length of the tarsus and longest forward-pointing toe together (excluding the nail). Before the emergence of the wing feathers, the standard wing length measurement, from the carpal joint to the ends of the primary feathers could not be taken. Instead the length of the terminal wing section was measured, including the metacarpal and phalanx bones (hatchling wing length). After feather emergence, the conventional wing length measure was also taken (standard wing length), both measures then including feather lengths. Chick biometrics were taken every two days.

When the first egg hatched in a nest the 'high protein' diet was provided so that diet would not restrict the growth of the chicks, because I wished to measure the after effects of the egg stage of development. This also allowed the chicks to grow into strong, healthy adults that had all experienced the same conditions after hatching. The diets of great tits (*Parus major*) and zebra finches as chicks have been shown to affect the clutch sizes they produce as adults (Haywood and Perrins, 1992). A consistent chick diet would therefore allow the use of these birds in later experiments. Each chick was labelled using coloured nail varnish on one toe nail. The chicks were measured for 4 weeks following hatching, after which time growth becomes very slow. Final measurements of chick size and mass were taken approximately two months after hatching to detect any changes during the previous month of slow growth.

Second experimental trial

A second trial was conducted with another 23 pairs of birds to separate the effects of egg quality from those of female incubation behaviour. The procedure was the same until laying began. To test whether parental incubation behaviour was affected by the treatment, for 21 clutches eggs that were laid on the same day were swapped between the two treatments. In this way each female was allowed to incubate the same number of eggs as she laid, but HQP females sat on eggs laid by LQP birds and vice versa. Each egg was recorded as successfully hatched or not. I was then able to compare the hatching success of eggs laid by females from each treatment when they were incubated by the laying female (the previous experiment in this chapter) and when they were incubated by females of the other treatment (this experiment). The relative effects of pre-laying diet on maternal behaviour and egg quality effects on hatchability could then be compared.

The two experiments provide eggs from four treatment groups, depending on whether they were produced and/or incubated by HQP or LQP females. The procedure for each of these groups is summarised below (HP=high protein diet, LP=low protein diet, n=number of clutches).

			n
HP diet - pair - LP diet (lay)	⇨ HQP incubators	⇨ HQP eggs, unswapped	32
	⇘ ⇗	⇘ LQP eggs, swapped	10
	⇗ ⇘	⇗ HQP eggs, swapped	11
LP diet - pair - LP diet (lay)	⇨ LQP incubators	⇨ LQP eggs, unswapped	24

Data analysis for hatching success

Most clutches were either 'successful' (most eggs hatched) or a 'failure' (hatching was unlikely for any of the eggs). The use of the 'proportion hatched' would have created difficulties in the interpretation of the results of an analysis for treatment effects because clutch size varied between 1 and 7 eggs. The proportion hatched from a one egg clutch must fall into one of only two categories, either 0% or 100%, whereas a 7 egg clutch may fall into one of 7 categories. Each clutch size therefore provides a different set of categories, so comparisons are difficult among clutch sizes. There were not enough clutches of any particular sample size to analyse one clutch size at a time and the modal clutch size was different for the two treatments. A G-test was therefore used to determine the overall effect of female diet prior to laying (treatment) on hatching success (at least one egg hatched) or failure (none of the eggs hatched). Logistic regression was employed to determine the factors responsible for the variation in hatchability. This is designed for data with a dichotomous dependent variable, here the hatching success or failure for each clutch, as used in the G-test. This approach makes sense for this data; in most cases a majority of the eggs in a clutch hatched or none at all. I pooled the data for both trials to compare swapped with unswapped clutches.

Results

Hatching success

A G-test indicates that unswapped clutches from HQP females were more likely to include eggs that hatched than those from LQP birds ($G=4.820$, $df=1$, $P<0.05$). 25 out of 32 HQP birds were successful in hatching chicks, but only 9 out of 24 LQP birds. None of the 17 eggs of less than 0.85g hatched. Of these no signs of development were found in those of less than 0.8g, but many other eggs developed to a great extent before embryo death.

There is therefore a treatment effect on hatching success which could have occurred for three reasons. Firstly, if light eggs are less viable than heavy eggs, the significant difference in egg mass between treatments could have caused the difference in hatchability. Secondly, there may be other differences in egg quality that are not reflected in mass. Thirdly, the treatment may affect parental behaviour either by lowering the capacity of LQP birds for incubation, as a result of body reserve depletion, or because a female may know when she has laid eggs with a low likelihood of hatching, and may not invest resources in their incubation. These three possibilities can be tested.

A Wilcoxon matched-pairs signed rank test was used to determine whether egg hatchability was related to egg mass within clutches. In 14 unswapped clutches in which some eggs hatched, but some did not, the eggs that hatched were heavier than the unhatched eggs ($Z=-2.166$, $P=0.030$). The medians were 1.075g for eggs that hatched and 0.981g for those that did not.

Logistic regression was used to make a fuller analysis of the effects of the following variables on hatchability: clutch size; mean egg mass; the treatment of the laying

female; and the treatment of the incubating female. Clutch means were entered in this analysis because between-treatment comparisons were made; individual egg data would not have been independent because treatment effects act through the laying hen, therefore each egg is a sample of the effect on the mother.

In chapter 4, a significant difference in egg masses between treatments was shown. If egg mass is related to egg hatchability then a treatment effect might act through a difference in egg masses. Alternatively, there may be variation in female incubation behaviour due to differences in the sizes of the body reserves remaining after egg production, or qualitative differences between the eggs of the two treatments. I therefore included the treatment of the laying female as a variable because this may affect egg quality, and the treatment of the incubating female, because it could affect incubation behaviour. By including the data from both unswapped and swapped clutches, I tested whether there was a significant amount of variation in egg hatchability. that is explained by either of these variables, after allowing for the effects of the other variables in the analysis, i.e. was one group of females more successful than the other in incubating eggs irrespective of the treatments of the laying females, and did either group lay eggs more likely to hatch than those of the other group, irrespective of the treatments of the incubating females?

Clutch size could also influence hatching success. The high energy costs of incubating a large clutch and the difficulty in making contact between each of the eggs and the brood patch could cause a negative relationship. Any correlation between clutch size and hatchability must be taken into account in the analysis of the other variables. The exchange of eggs between clutches necessitates the use of two clutch size variables: the number of eggs laid in the original clutch and the number of eggs in the incubated clutch. For unswapped clutches these were equal, but they differed for many swapped clutches.

All of the variables were entered into a single logistic regression in one step. Table 5.1 shows that mean egg mass and the clutch size laid explained significant amounts of the variation in hatching success after allowing for each of the other variables. There was no evidence for a negative correlation between clutch size and hatchability, as postulated, instead there was a positive correlation (see the R values in table 5.1 for the partial correlations). With only mean egg mass and the clutch size laid in the analysis a highly significant model was produced (χ^2=26.200, df=2, P<0.0001). There was no significant deviation of the regression from a perfect fit (χ^2=77.839, df=74, P>0.05).

Egg mass, chick size and condition

An index of hatching body size was produced by principal components analysis of three measures (head and bill length, wing length, and tarsus and toe length). One factor explained 75% of the variation in the data. This was correlated with the mean egg mass of the eggs that hatched (figure 5.1). Hatched chick mass, measured before the first feed, was related to mean egg mass (figure 5.2) in both treatments. However, "mass" includes variation in both body size and condition. Hatched chick mass was therefore regressed against body size, showing a strong relationship (figure 5.3), the results of which gave a body condition score. Body condition was not related to egg mass (figure 5.4). The above results were pooled after analyses of covariance showed no differences in the gradients or elevations of the regression lines, indicating the lack of any treatment effect, after allowing for variation in egg mass.

The mean egg mass laid differed significantly between treatments (HQP mean=1.059g, SE=0.016, n=43; LQP mean=0.986g, SE=0.018, n=34; t-test t_{75}=3.03, P=0.003), as in chapter 4. A relationship between egg mass and chick size at hatching (an index of body size produced from a principal components analysis) has been demonstrated. I would therefore expect that chick size at hatching would differ

between eggs from the two treatments, but this was not so: there was no difference in chick size between treatments (HQP mean=-0.002, SE=0.281, n=17; LQP mean=-0.363, SE=0.282, n=8; t-test t_{23}=0.79, P=0.437). This was because the mean masses of the eggs laid was different in the two treatments, but the mean masses of those that hatched was not, i.e. the smallest eggs did not hatch (HQP mean=1.103, SE=0.102, n=16; LQP mean=1.044, SE=0.076, n=9; t-test t_{23}=1.50, P=0.147)

Chick growth

The effect of treatment on chick growth was tested using the Genstat statistical package. The growth curves are shown in figures 5.5 to 5.9. Growth models - logistic, Gompertz, and Von Bertallanffy - were fitted for each measure. The best-fitting model in each case was the Gompertz model. The residuals were calculated for each measurement of each chick so that linear modelling could be applied: the absolute residuals were regressed against age. This produced a function describing the expected residuals for chicks of any particular age. To control for the difference in the magnitude of the residuals for chicks of different sizes, since large chicks show greater differences in size than small chicks, the residuals were divided by the expected values for a given age i.e. proportional values were used. The sign of each residual was retained. The treatment acted on the laying female, therefore effects on the eggs or chicks were tested at the clutch level, not the individual egg or chick level. Also, the data so far represented multiple measurements for each across a time sequence. The data are therefore not independent. To control for these two effects, mean values were calculated for the chick-day values of each clutch. This also controlled for the differential growth rates shown to occur between the largest and smallest nestlings in zebra finches (Skagen, 1988). The 1634 observations were therefore condensed to 27 usable values. Analysis of variance was used to test whether the variance within each treatment was greater than the variance between the

two treatments. There was no evidence for any effect of treatment on growth rate (table 5.2).

The growth curve in figure 5.5 shows that after an initial fast phase of growth there was a brief period of decreasing mass. This occurred between day 13 and day 18 after hatching. The graph shows measurements taken every 2 days. The chicks did not become skeletally smaller, so the heaviest chicks used up some of their reserves at this time.

The percentage of chicks surviving to fledge was not related to the mean egg mass of the eggs that hatched in a treatment (HQP chicks - Spearman's r_{19}=0.215, P=0.377; LQP chicks - r_9=0.069, P=0.861), neither was there a difference in survival between treatments (Mann-Whitney U test, U=64, Z=-1.203, P=0.229). Of 19 clutches hatched by HQP birds and monitored during chick rearing, 9 included chicks that died within two days of hatching. Of 9 clutches hatched by LQP birds, two had deaths within two days of hatching.

A principal components analysis of body size measurements at fledging was based on head and bill length, wing length, and tarsus and toe length. This produced one factor which accounted for 57% of the variation in the data. Body size at fledging was positively correlated with the mean mass of the eggs that hatched for chicks from HQP birds, but negatively for chicks from LQP birds (figure 5.10). However, all but one of the data for chicks from LQP birds lay within the same range as that for the chicks from HQP birds, and this datum had a disproportionate effect on the correlation. The removal of this datum did not produce a significant correlation (r_7=0.006, P=0.990) for LQP birds, but the sample size was very small. A larger sample of LQP broods is required to test whether there is a correlation between egg mass and body size at fledging for LQP birds.

The overall effect of the pre-laying dietary treatment on egg formation, incubation and rearing was that a mean 2.67 young per clutch (SE=0.44, n=27) were fledged by HQP birds, whilst only 0.85 young per clutch (SE=0.27, n=27) were fledged by LQP birds (t_{43}=3.53, P=0.001).

Table 5.1- Table of results for a logistic regression of five variables on the hatching success (at least one egg hatched) or failure (none of the eggs hatched) of clutches. All of the variables were entered at the same time. The regression coefficients (B) and their standard errors (SE) are given. Their significances were tested using the Wald statistic, the squares of the ratios of the regression coefficients to their standard errors for variables with one degree of freedom. This has a chi-square distribution. The R statistic, used to look at the partial correlations, ranges between +1 and -1. A positive value indicates a rise in the value of a variable with an increase in the probability of hatching success (note treatment variables are categorical).

Variable	B	SE	Wald	df	Sig	R
Mean egg mass	8.76	3.64	5.78	1	0.016	0.200
Treatment of the laying female	2.06	1.19	3.01	1	0.083	0.104
Treatment of the incubating female	-1.93	1.23	2.47	1	0.116	-0.070
Clutch size laid	1.31	0.64	4.24	1	0.039	0.154
Clutch size incubated	-0.34	0.64	0.27	1	0.601	0.000
Constant	-12.83	4.23	9.19	1	0.002	

Table 5.2 - Results of an analysis of variance to test whether the pre-laying dietary treatment of females affected the growth of the chicks that they subsequently produced. The mean residual of a non-linear regression was calculated for each clutch. Differences in the magnitude of residuals for chicks of different ages were taken into account. Table F with 1 and 25 degrees of freedom is 4.2417 (P=0.05), and with 1 and 24 degrees, 4.2597 (P=0.05).

Mean corrected residuals	F	Significance
Mass	$F_{1,25}$=3.0053	NS
Head & bill length	$F_{1,25}$=0.2181	NS
Hatchling wing length	$F_{1,24}$=3.4033	NS
Standard wing length	$F_{1,25}$=0.5117	NS
Tarsus & toe length	$F_{1,25}$=0.0517	NS

Figure 5.1. Correlation of the clutch means for chick size on the day hatched (before the first meal) and egg mass for those that hatched.
r=0.729, P<0.001, n=23

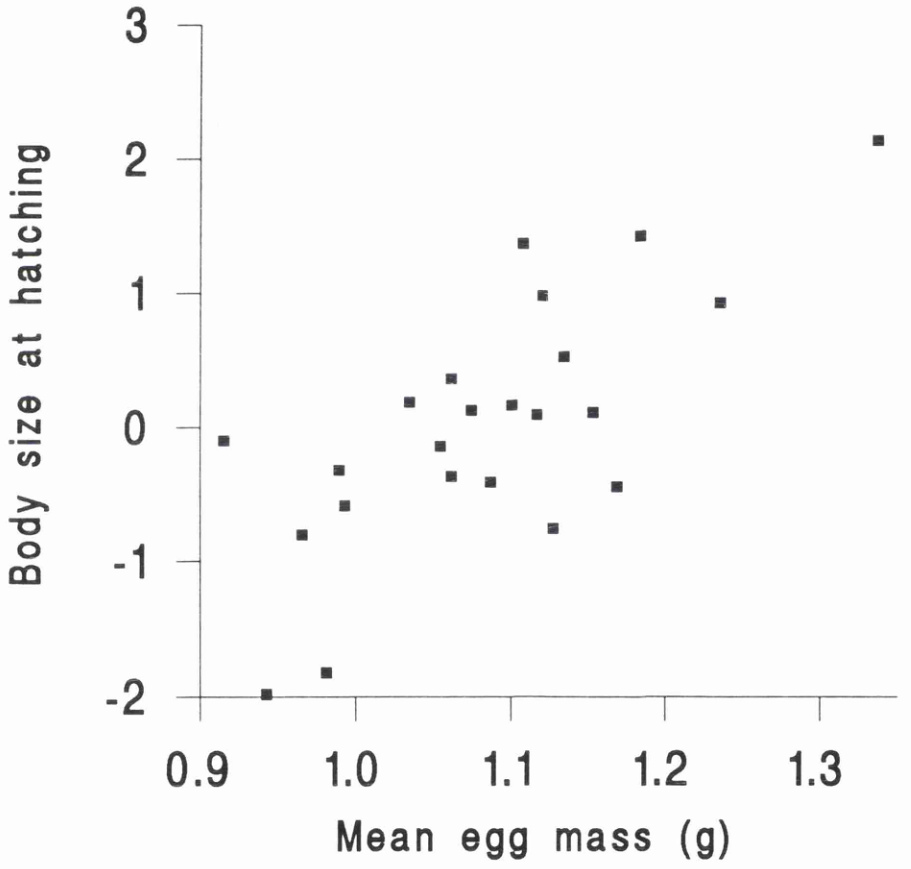


Figure 5.2. Correlation of the clutch means for chick mass on the day hatched (before the first meal) and egg mass for those that hatched.

Pearson's r=0.699, P<0.001, n=25

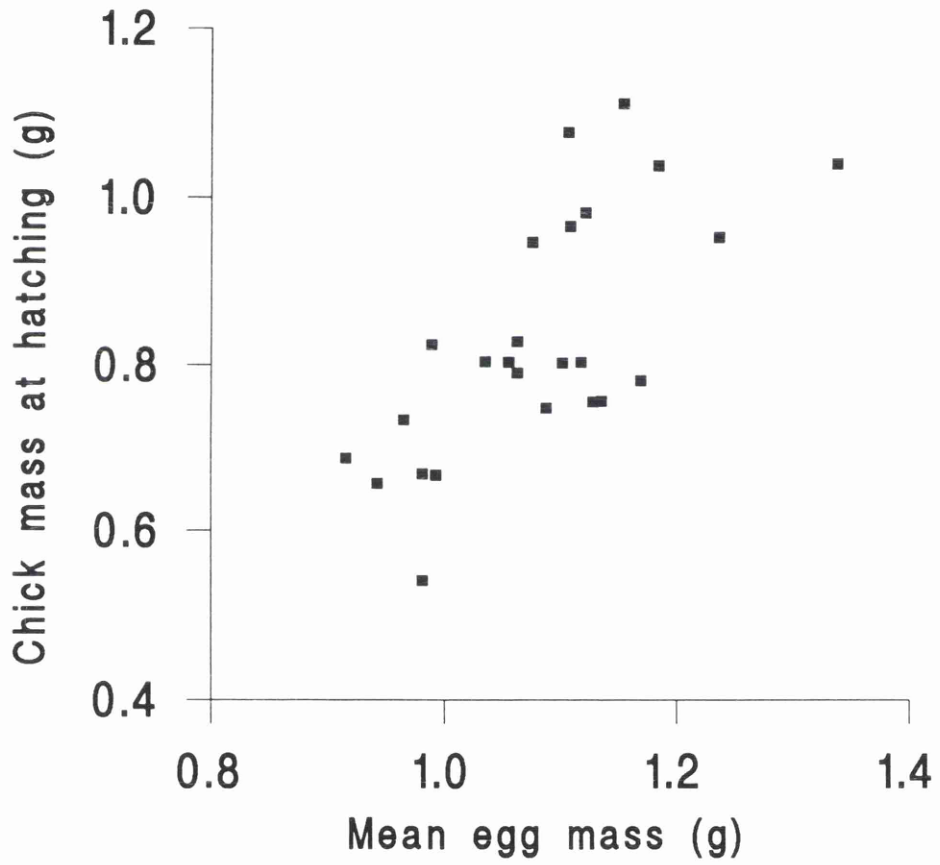


Figure 5.3. Body condition on the day a chick hatches, was derived as the residual of the regression of body size on egg mass. The clutch means for hatched eggs were used.
r=0.804, P<0.001, n=25
y = 0.83540 + 0.09694x

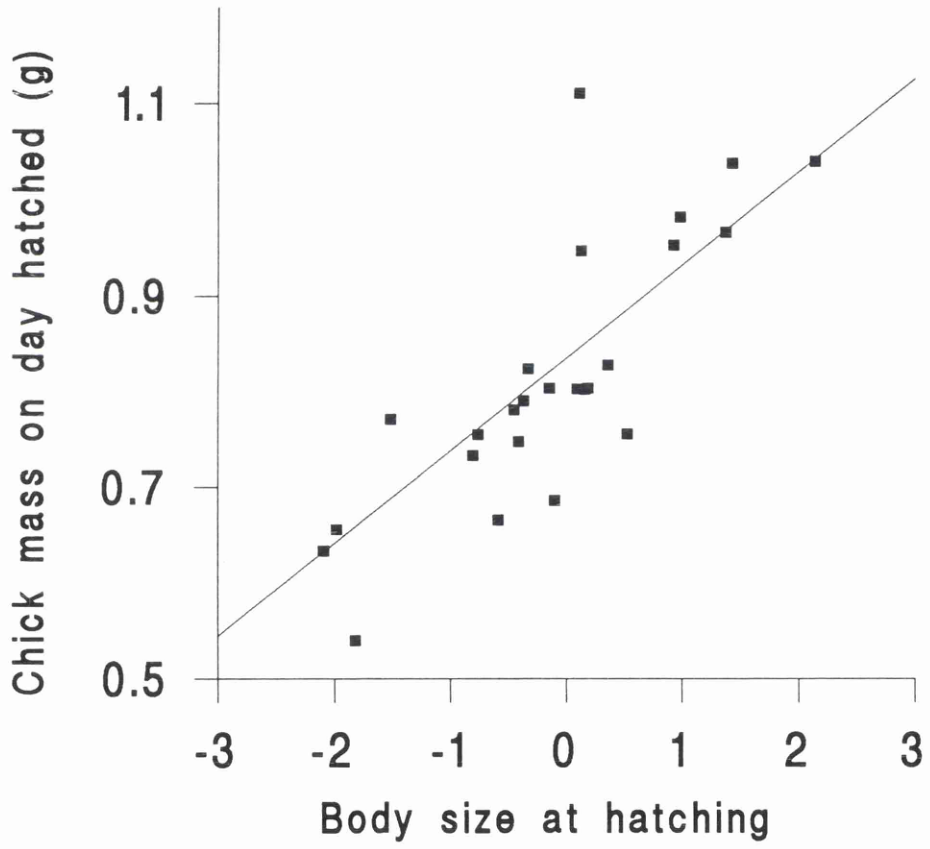
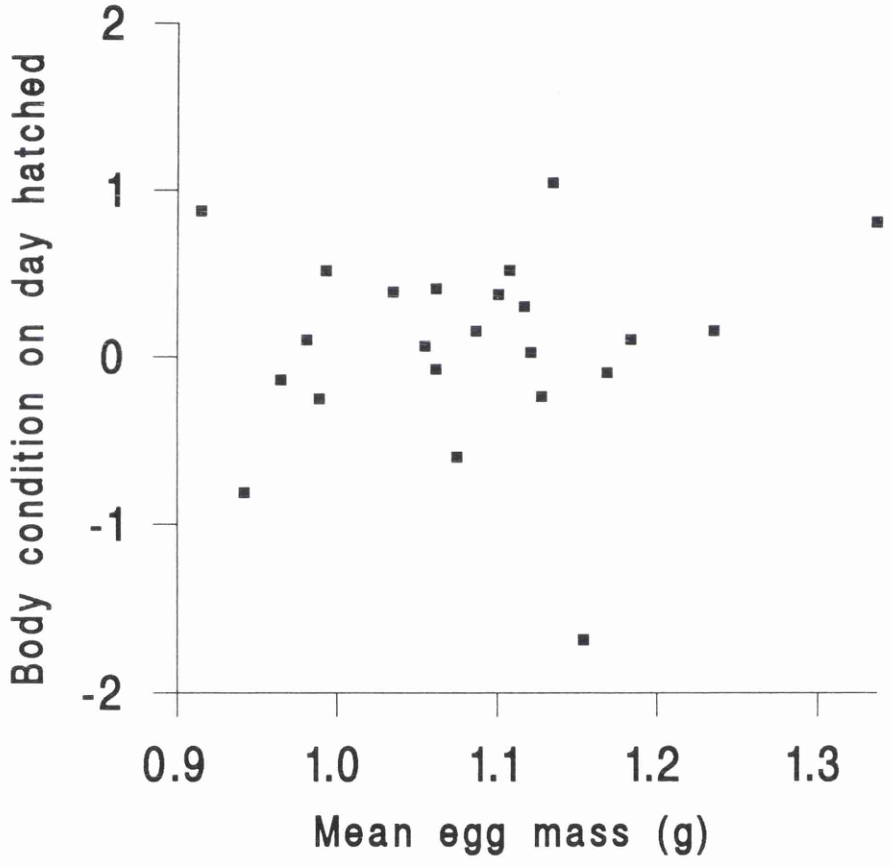


Figure 5.4. Correlation of the clutch means for the body condition of chicks on the day hatched and egg mass for those that hatched.
r=0.293, P=0.175, n=23



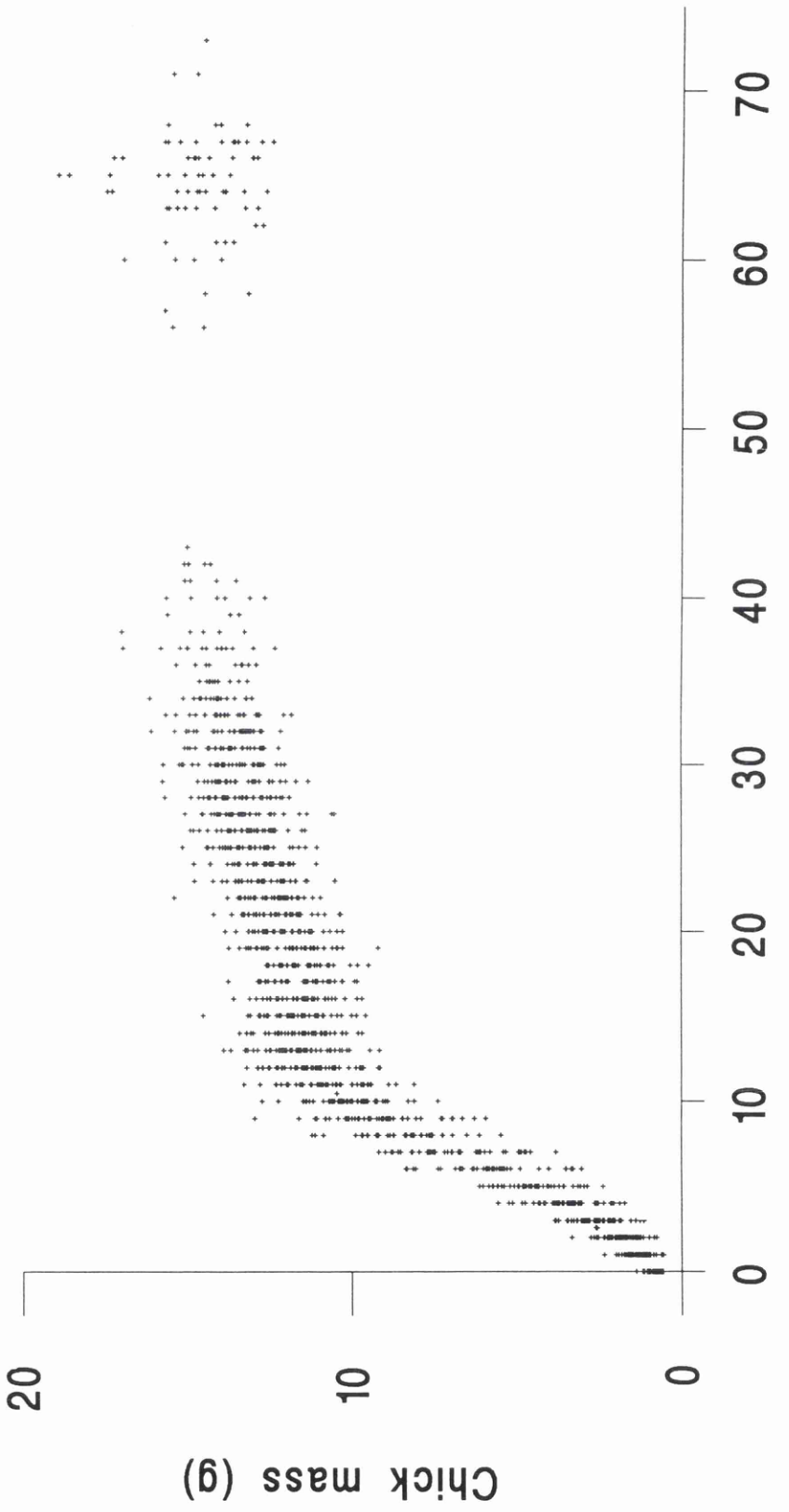


Figure 5.5. Growth curve for the masses of chicks, measured every two days from hatching to fledging. When growth slowed greatly, measurements were postponed for one month, when the chicks had fledged.

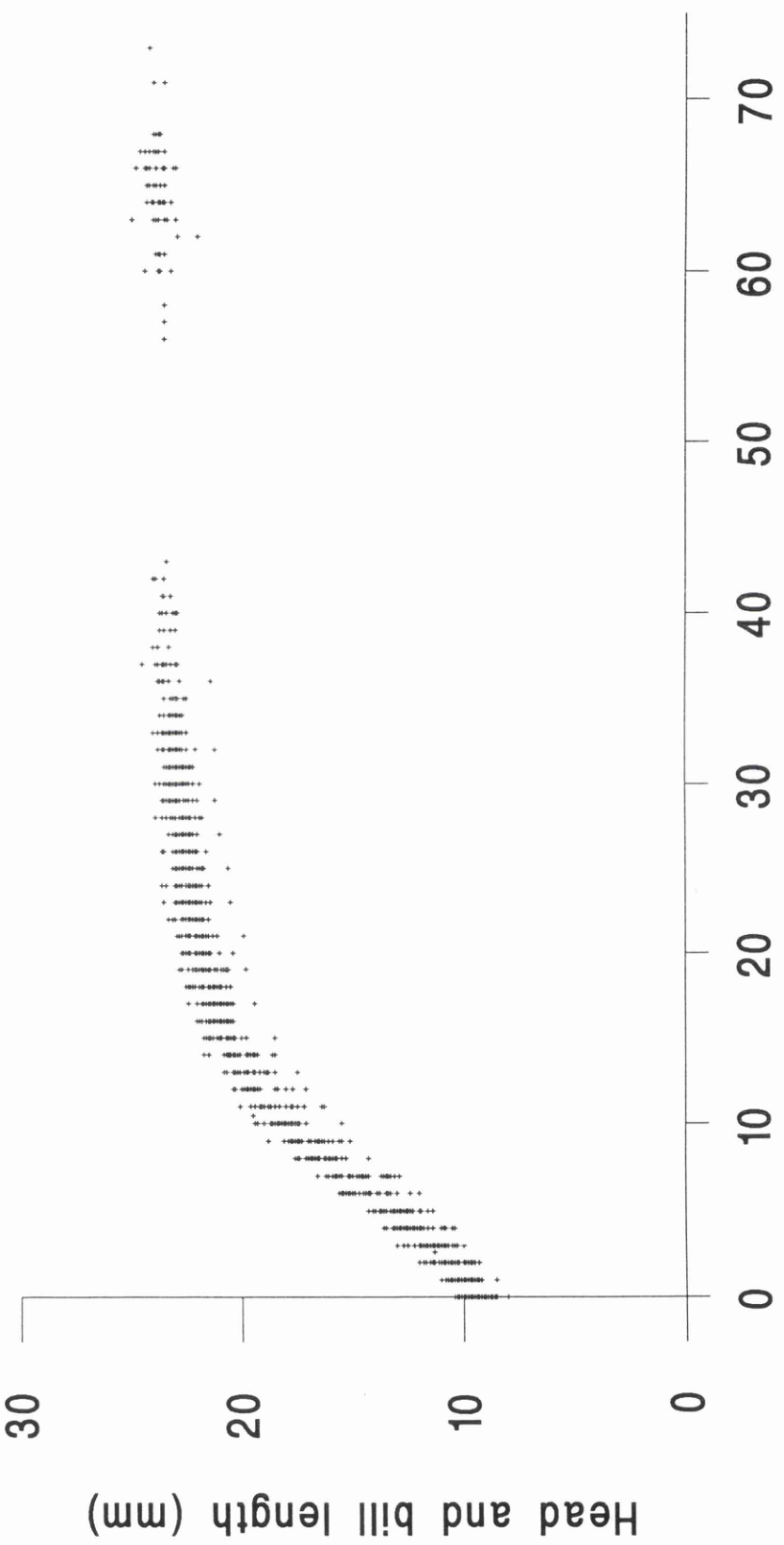


Figure 5.6. Growth curve from hatching to fledging, for the lengths of the head and bill, measured every two days. When growth slowed greatly, measurements were postponed for one month, when the chicks had fledged.

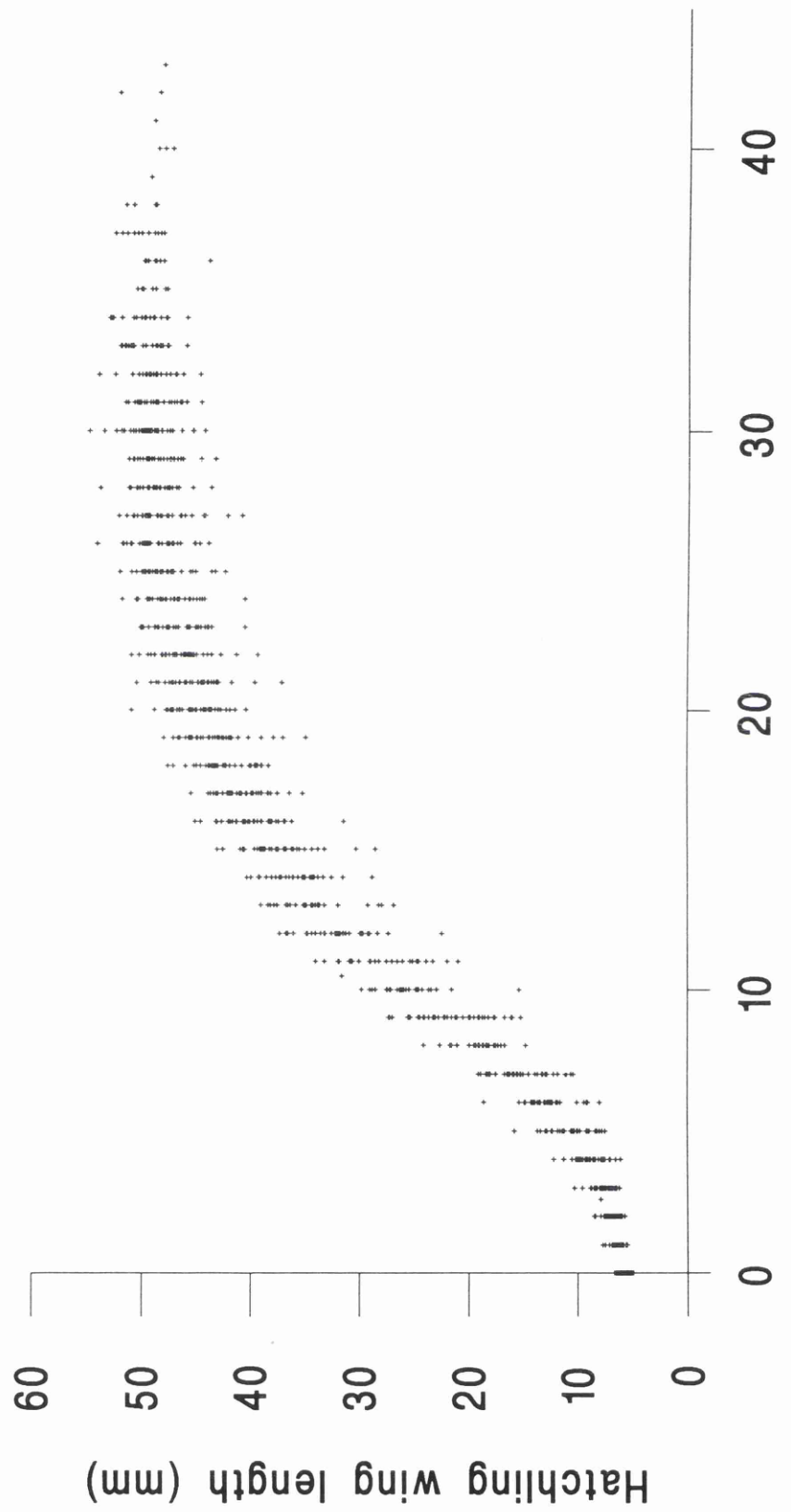


Figure 5.7. Growth curve from hatching to fledging, for "hatchling wing length" (the length of the metacarpal and phalanx bones), measured every two days.

Figure 5.8. Growth curve from hatching to fledging, for "standard wing length" (as normally measured on an adult bird), measured every two days. When growth slowed greatly, measurements were postponed for one month, when the chicks had fledged.

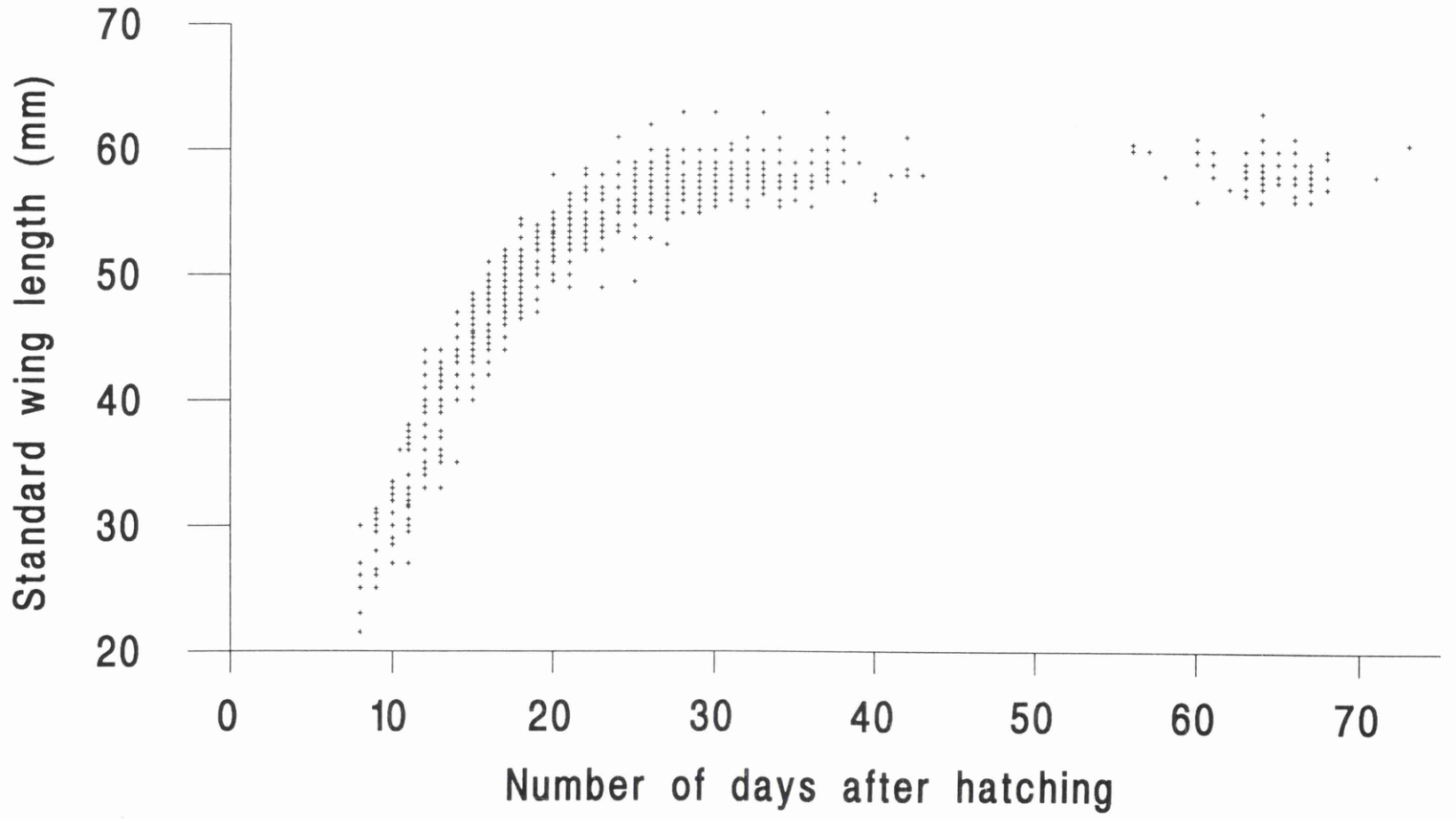


Figure 5.9. Growth curve from hatching to fledging, for the length of the tarsus and the longest forward-pointing toe, measured every two days. When the growth slowed greatly, measurements were postponed for one month, when the chicks had fledged.

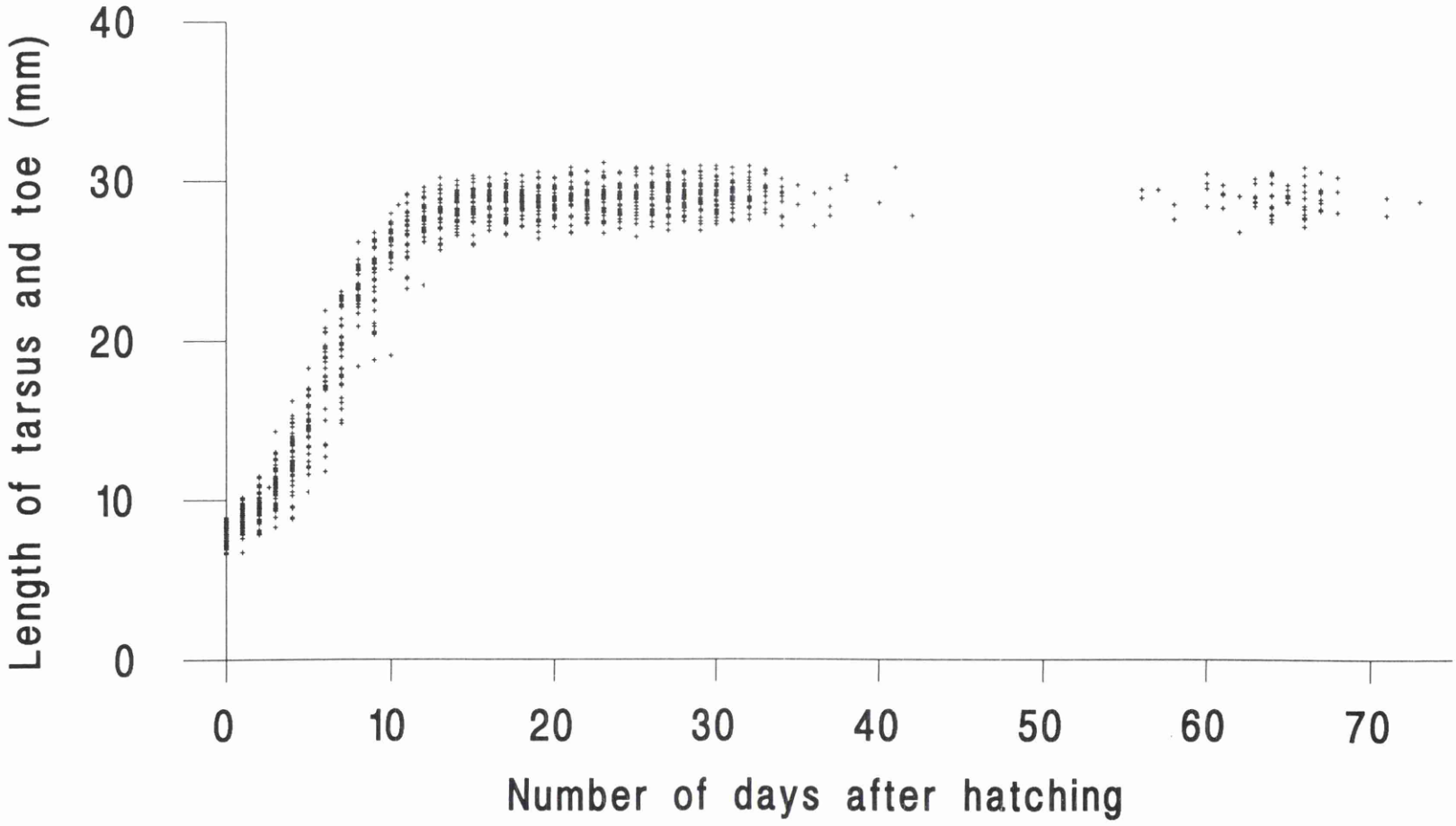
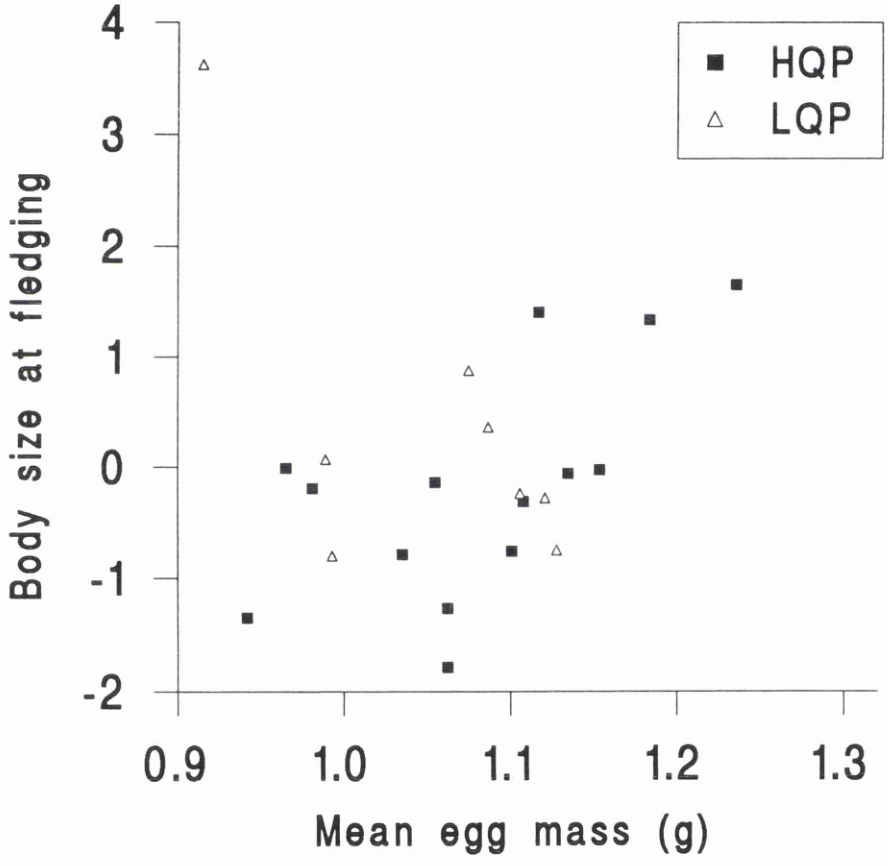


Figure 5.10. Correlation of the clutch means for chick size at fledging and the egg mass of those that hatched. The layers were from two pre-breeding dietary treatments, giving females of "high quality protein" (HQP) or low (LQP).
HQP: r=0.669, P=0.006, n=15
LQP: r=-0.661, P=0.074, n=8
When the outlier is removed
r=0.006, P=0.990, n=7



Discussion

Hatching success

Hatching success varied widely among clutches, but most clutches were either successful in hatching most of the eggs, or else failed completely. The LQP birds were so unsuccessful that only 9 clutches from 24 hatched. Three hypotheses explaining why egg hatchability might have varied between pre-laying dietary treatments were tested. Firstly, the treatments varied in the masses of the eggs that were laid. This could itself affect the viability of the eggs. Secondly, there may be other effects of the treatments that might show as differences in egg quality i.e. there may be variation in the contents of the eggs that might not be correlated with the variation in egg mass. Thirdly, the variation in the quality of a female's protein reserves may affect her behaviour during incubation.

Clutch size was included in the analysis because a negative relationship with hatching success might be expected if a high amount of energy were required to incubate a large clutch, causing energetic stress to the incubator, or there were difficulties in covering the eggs. However, there was no evidence that the zebra finches had any problem in providing the heat required for incubation. The clutch sizes observed were therefore below the level required to have any effect on hatching success under the favourable and constant conditions provided for this captive population. In fact, clutch size was positively related to hatching success. This relationship is independent of any treatment effects, but is only significant for the sizes of the clutches laid, not the clutches incubated. This indicates that the effect is due to the influence of the laying bird, via its endowment of nutrients and a protective shell covering, not the minimisation of heat loss when lying in a nest with many other warm eggs. One possibility is a correlation between clutch size and egg quality, irrespective of female treatment. This could produce the significant relationship observed between clutch

size and hatching success whilst not producing a treatment effect via egg quality, as indicated by the variable "treatment of the laying female".

Hatching success was also related to egg mass, heavy eggs standing a greater chance of hatching than light eggs. Any variable that affected egg mass would, therefore, have affected the likelihood of an egg hatching. Since the pre-laying diet of a female may affect egg mass, HQP females laying heavier eggs than LQP birds (chapter 4), an indirect effect on egg hatchability is implied. Since egg mass was not related in any way to the levels of protein reserves in a laying female a reserve quality effect is indicated. This is not likely due to variation in lipid quality because HQP birds, on average, increased their body condition during laying, whilst protein condition declined. This rise in body mass must have been due to an increase in fat levels. If lipid quality was important I would have expected a decline in lipid reserves, in order to release those essential fatty acids required in egg production. This did not occur, implying that reserve effects were due to protein reserve quality. The diet of a female prior to breeding can therefore affect egg hatchability through variations in the quality of body proteins. It is worth noting that there may be a correlation between a bird's productivity in egg production and its success in hatching the eggs. This is commonly termed a "parental quality" effect (Bolton, 1991; Williams, 1994). To allow for this the eggs would have to be exchanged between clutches within each treatment. This was not attempted. However, assuming that my results are not purely an effect of parental quality, these results are in agreement with those of Perrins and McCleery (1994) who have correlated egg mass with the probability of an egg hatching in the great tit.

If the quality of a female's boy reserves can affect egg hatchability through variation in egg mass, as proposed above, egg quality (the constitution of an egg after allowing for egg mass) might also be linked with hatching success. If this occurred, I would have expected a relationship between the treatment of the laying female and the

hatching success of her eggs, irrespective of the treatment of the incubating bird. However, there was no relationship after allowing for mean egg mass and clutch size. Also, any effect of female incubation behaviour would be indicated by a relationship between the treatment of the incubating female and hatching success. Again, no effects, independent of clutch size and mean egg mass, were observed. There is therefore no evidence here for any effect of egg quality or female incubation behaviour on hatching success, but that does not mean that they did not occur to any degree. Such effects may be masked by clutch size. In chapter 4 a significant difference in clutch size between treatments was demonstrated. High clutch sizes may therefore correlate with other successful attributes for hatching, such as the potential effects of treatment on incubation behaviour or egg quality. These possible masked effects cannot be tested with this data.

The LQP birds in this study produced many eggs and yet very few chicks hatched. I find this surprising since one would expect that there would be selective pressure to prevent breeding when nutritional constraints cannot allow the production of viable eggs. This may be a result of generations of captive breeding. There may be no cost to producing inviable eggs, in terms of the number of offspring surviving to reproductive age in a captive colony. Alternatively, the control of breeding and the number and size of eggs produced may not be related to the nutritional state of a bird, i.e. eggs can be laid when a bird is not in a state that would allow any degree of success. It could be that the control mechanism is not affected by protein quality, but possibly by the size of the protein reserve. In the wild, perhaps birds rarely have both large protein reserves and poor protein reserve quality and therefore the overall size of protein reserves could be used to gauge the amount of high quality protein a bird has stored in its body. There is no information regarding the protein condition of wild zebra finches and the quality of reserves has only recently gained the interest of researchers (see Bolton, 1992, 1993). I would suggest, however, that protein quality is very important in reproductive success and deserves a great deal of attention to determine how

important it is in determining reproductive success, and what part it may play in the control of reproduction. The size of the protein reserve, however, does not appear to relate to the timing of laying. Body condition, however, does. Perhaps the energetic reserves of a bird are involved in the decision to breed or not, but not protein reserves. A bird might therefore breed when it is in good body condition, even though it may have very low levels of the proteins required in egg formation. Eggs might be laid before the production of inviable eggs can be halted. The physical stimulus of the eggs on the brood patch appear to cause the disruption of follicle growth in the zebra finch during the second and third days of laying (Haywood, 1993). A number of inviable eggs might therefore be produced where there is protein available, even though the specific amino acids required to produce a high quality egg are not available.

Egg mass, chick size and condition

The question of whether egg mass affects the hatching size or condition of chicks has recently been reviewed by Williams (1994). Here I use the term egg mass to include both the mass and size of eggs, since these are very closely correlated (Hoyt, 1979). Williams states that of 38 species studied, 35 showed a correlation between chick mass at hatching and egg mass, and of those that also showed a correlation between egg mass and chick size, the chick mass relationship was stronger in all cases. However, this does not show that condition was affected more than size, because mass is affected by both body size and condition, so if egg mass affects both hatchling size and condition to some degree, the relationship with chick mass will be stronger than either of them, since it will show the combined effects.

To compare chick condition with size, the analysis of chick mass should allow for body size variation. Body condition can be produced as the residuals of the regression of mass on body size (see chapter 2). Very few studies have done this: of the four that

have, three have concluded that egg mass affects the body reserves of chicks to a greater extent than their size (Birkhead and Nettleship, 1982; Jarvinen and Ylimaunu, 1984; Reid and Boersma, 1990). O'Connor (1979), however, concluded the opposite for the European swift, *Apus apus*. Studies of condition have so far mainly used a single measure to scale body size, but an overall "body size" measure would be more appropriate. The results of correlations between egg mass and chick size have therefore varied within studies on single species, depending upon which part of the body was measured, for example Rofstad and Sandvik (1987) found a correlation between egg mass and the wing and head measurement of hooded crow chicks, *Corvus corrix*, but not with tarsus length. A measurement of overall body size is required; this can be produced by principal components analysis of a number of body measurements.

My conclusions were the same as those obtained by O'Connor for the common swift: egg mass was related to the size of the chicks at hatching, but not their body condition. These are therefore in contradiction to the general theory at present, though the evidence for passerines is sparse (Williams (1994) uncovered only 6 studies of passerine species). The use of mass as an index of body condition is misleading because no conclusions regarding body condition can be drawn from such an analysis, for instance, in this study of the zebra finch, chick mass correlated strongly with egg mass and yet, after allowing for body size, no relationship was found for body condition. The results of analyses based on mass data, with no control for body size (the bulk of the evidence) cannot be considered as good evidence for a relationship between egg mass and body condition.

In this study zebra finch egg mass was correlated with chick size, but not chick condition. This implies that the rate of growth or the timing of hatching depend on the size of the reserves within the egg, but body condition does not relate to egg reserve size when laid. Body reserves were therefore either not related to egg mass, or the

chicks hatched with a specific level of reserves as an insurance against starvation within the first few days after hatching. Variations in chick size must therefore be caused by either the rate of development within the egg, or the timing of hatching.

Egg mass was lower for LP birds than for HP birds (chapter 4), but this did not carry through to any effect of the treatment on chick size at hatching. This was because there was an effect of egg mass on egg hatchability: the lightest eggs did not hatch. The masses of the eggs that did hatch were not significantly different between the two treatments. There was therefore a relationship between egg mass and chick size but this did not differ between treatments, nor was there a large difference in the range of egg masses that hatched in the two treatments. No evidence was found for any treatment effect on chick size, body condition or survival, following hatching. Survival was not affected by egg mass either. Further study would be useful to determine the factors that have brought about an egg mass-chick size relationship in two known species, but an egg mass-chick condition relationship in three others, and possibly many more.

Growth

The effect of egg mass on chick size was retained through to fledging for HQP birds. If a bird hatches when small, this could possibly affect it in later life, since fledging size has been linked with a bird's ability to survive to breeding age (Perrins, 1965). There were no treatment effects on hatching size or condition or growth rate. However, treatment differences would be unlikely to be shown for the chick data because the effects of treatment on egg production and hatching success produced a large number of HQP chicks, but only very few LQP chicks. There was, however, no indication of any separation of the growth curves for chicks from HQP and LQP birds. The combined effects of the pre-laying diet on egg production and egg hatchability are therefore biologically much more significant than any possible effect

on growth rate. It might be worth collecting more data for LQP chicks, taking fewer measurements from many more nests. If a significant treatment effect on chick growth were found this could be mediated through either the quality of the egg from which the chick hatched, or carry-over effects on the ability of the adult to provide for the chicks. These results are ecologically meaningful - they lump all the effects of treatment on chick growth rate - but they are less illuminating physiologically because they do not separate the mechanisms of the effect. If understanding the mechanism is important, the effects could be separated by moving eggs between nests with different treatments. I believe, however, that running the experiment again would be unlikely to affect the results.

In summary, the diet of a female prior to breeding, and hence the state of her body reserves, was related to hatching success, most likely as a result of variation in egg mass. The number of young fledged by HQP birds was nearly three times that fledged by LQP birds. Chick size at hatching was correlated with egg mass and this relationship might be retained through to fledging, providing the possibility of ongoing effects in the juveniles. Pre-laying diet did not, however, affect chick size or growth.

Chapter 6 - Final Discussion

Many studies of the effect of condition on reproduction have measured body mass in relation to body size. It is now, however, practical to measure protein and fat reserves using one of the methods recently developed: ultrasound (Sears, 1988), total body electrical conductivity (Walsberg, 1988), or muscle profile (Bolton, Monaghan and Houston, 1991; see chapter 2 for a method for use with small birds). In order to use the muscle profile methods for a new species, a sample of carcasses is required with which to set up the estimation equations. A more accurate assessment of body condition might be possible once the biochemical nature of animal reserves has been identified. Houston, Donnan, Jones, Hamilton and Osborne (in press) have demonstrated the loss of a specific protein of high molecular weight during egg formation in zebra finches. This could form a muscle protein reserve of specific nature, as proposed by Kendall, Ward and Bacchus (1973). Future studies might determine whether this protein is found in other species and similarly used as a reserve. Identifying this protein might allow the direct determination of the level of protein reserves in a bird by muscle biopsy.

In the introduction to this thesis I proposed that the level of a protein reserve might limit the number of eggs that a female can produce. The level of the protein reserves of red-billed queleas plays a role in determining clutch size. The availability of food has been shown to affect clutch size (Arcese and Smith, 1988; Perrins and McCleery, 1989), and egg mass (Nisbet, 1978; Perrins and McCleery, 1994). The level of reserve protein has also been shown to affect clutch size (Jones and Ward, 1976; Bolton *et al.*, 1993). However, no relationship was found between the protein status of a zebra finch and its subsequent reproductive performance. As a result, I looked at the effect of reserve quality on egg production. I demonstrated that the quality of the diet of a zebra finch prior to egg formation does not only affect the number of eggs subsequently laid but also egg mass and hatchability. This was most likely due to the addition of egg proteins to the pre-breeding diet. The result was that birds that

received a high quality protein diet raised many chicks successfully, whereas those that received a diet of low quality protein raised very few, even though both groups were provided with an identical seed diet during egg formation. Both groups of birds had similar levels of body protein both prior to and following egg formation.

Reserve quality is therefore an important factor in dietary studies. Merely analysing the levels of protein or fat in an animal may not show some important changes in body reserves. The use of reserves therefore cannot be ruled out on the basis of a lack of any change in the levels of fat or protein. More specific changes may be occurring. In order to take proper account of this the identity of a nutrient reserve must be known. Species may vary both in their reserve requirements and the selective advantage to be gained by their deployment. This is likely to relate to the dietary ecology of a species. Those that have a protein-rich diet are more likely to be limited by their intake of fat or carbohydrate, for instance many waterfowl (Ankney and Alisauskas, 1991), whereas herbivores, such as the granivorous zebra finch, may have difficulty finding the protein requirement at certain times. Studies of other species may reveal interesting comparisons of reserve use and dietary ecology between species.

This thesis has addressed the possible relationships between egg production and the use of body reserves by zebra finches. Haywood (1993a) studied the mechanism of control of clutch size in this species. The cessation of egg laying was triggered by the tactile stimulation of the eggs on the breast of a bird. Stimulation by only one egg was required to lay a normal clutch, although this species is an indeterminate layer (Haywood, 1993b). Laying appeared to be stopped by the disruption of follicular growth during the third or fourth day of laying. Most birds laid four to six eggs, the clutch size produced therefore depending on the number of follicles that continued to mature and ovulate after follicle disruption and the number that atrophied and were resorbed.

The timing of follicular disruption therefore did not account for the variation in clutch sizes, as it can for blue tits (Haywood, 1993c). In kestrels, *Falco tinnunculus*, (Beukeboom, Dijkstra, Daan and Meijer, 1988), the number of eggs laid is closely related to laying date, late layers laying small clutches. The incubation tendency of a female is thought to increase through the breeding season. As a result, a female laying late in the season will be stimulated to incubate after laying only a few eggs, the follicular disruption thus caused resulting in a small clutch. Those that lay early begin incubating after a larger number of eggs have been laid, so the inhibition of egg formation, resulting from the stimulus of the eggs in the nest, is delayed causing a large clutch to be laid.

Studies of different species have therefore produced different results. Haywood (1993b) has reviewed the evidence so far, classifying birds as determinate, semideterminate or indeterminate layers, depending upon whether extrinsic factors, such as the number of eggs in the nest, can have an effect on the clutch size laid. The size of body reserves and food availability were suggested as factors relating to the evolution of determinate or indeterminate laying, as well as variation in the optimum time for rearing chicks. Indeterminate layers have evolved a flexible clutch size to allow for a wide variation in the conditions that determine the most productive clutch size. The determination of clutch size is delayed until after laying has begun. Determinate layers breed in environments in which the conditions affecting the most productive clutch size are unchanging, so the single most productive clutch size is laid. Females need only grow the follicles that will be laid.

Zebra finches are adapted to the very unpredictable environment of the Australian desert. The highly flexible approach of an indeterminate layer therefore allows this species to make the most of favourable breeding conditions by laying a large clutch when conditions are good.

At present, there is no evidence to suggest the mechanism that determines clutch size in the zebra finch. If the number of developing follicles continuing to mature during follicle disruption were affected by the nutrition of the bird, I would have expected either a relation between seed intake and clutch size, or between the pre-laying levels of body reserves and clutch size, assuming a reduction in reserves to a minimum level at the close of laying. Neither of these was observed in my study. I conclude, therefore, that protein reserves of a specific nature are required in egg formation, in proportion to the number of eggs laid, but that there was no evidence for any control of clutch size by overall protein reserves. There did appear to be some limitation of clutch size: birds lacking a reserve of high quality protein (low protein quality birds) on average laid four eggs and these were mostly small eggs of poor quality which did not hatch. There is evidence that the loss of egg viability may be the result of the low masses of these eggs. A clutch of four eggs could be considered as the normal "small" clutch size for a zebra finch since three eggs are usually laid before the disruption of follicle growth, a fourth then being ovulated to complete the clutch. Smaller clutch sizes may have been produced as the result of a lack of general protein reserves, since these birds had lost amounts of protein similar in quantity to those lost by the layers of the largest clutches amongst the birds with a reserve of high quality protein (HQP). It would be interesting to compare the clutch sizes and egg masses observed here with those found in the wild, but there is no such published data. Amongst HQP birds there was no relationship between clutch size and the level of body protein at the beginning or end of egg formation, indicating that clutch size was not limited by the size of the overall protein reserve. It is therefore possible that in HQP birds clutch size is controlled by hormone levels at the time of follicle disruption or the level of a specific unknown storage protein, the levels of which were not reflected in the muscle condition estimate used. There is great scope for a study of the endocrine and histological changes taking place during follicle disruption to discover the factors which ultimately determine clutch size.

References

Alisauskas,R.T. and Ankney,C.D. 1985. Nutrient reserves and the energetics of reproduction in American coots. *Auk* 102:133-144.

Ankney,C.D. and Alisauskas,R.T. 1991. Nutrient reserve dynamics and diet of breeding female gadwalls. *Condor* 93:799-810.

Ankney,C.D. and MacInnes,C.D. 1978. Nutrient reserves and reproductive performance of lesser snow geese. *Auk* **95**:459-71.

Arcese, P. and Smith, J.M.N. 1988. Effects of population density and supplemental feeding on reproduction in song sparrows. *J. Anim. Ecol.* **57**: 331-350.

Bancroft, G.T. 1984. Patterns of variation in size of boat-tailed grackle *Quiscalus major* eggs. *Ibis* **126**: 496-509.

Beukeboom, L., Dijkstra, C., Daan, S. and Meijer, T. 1988. Seasonality of clutch size determination in the kestrel *Falco tinnunculus*: an experimental approach. *Ornis Scandinavica* **19**: 41-48.

Birkhead, T.R. and Nettleship, D.N. 1982. The adaptive significance of egg size and laying date in thick-billed murres *Uria tomvia*. *Ecology* **63(2)**: 300-306.

Bolton,M., Houston,D.C. and Monaghan, P. 1992. Nutritional constraints on egg formation in the lesser black-backed gull: an experimental study. *J.Anim.Ecol.* **61**: 521-32.

Bolton,M., Monaghan,P. and Houston,D.C. 1991. An improved technique for estimating pectoral muscle protein from body measurements of live gulls. *Ibis* **133**:264-70.

Bolton,M., Monaghan,P. and Houston,D.C. 1993. Proximate determination of clutch size in lesser black-backed gulls: the roles of food supply and body condition. *Can.J.Zool.* 71:273-279.

Burley, N., Krantzberg, G. and Radman, P. 1982. Influence of colour-banding on the conspecific preferences of zebra finches. *Ethnology* **76**:133-151.

Castro,G., Wunder,B.A. and Knopf,F.L. 1990. Total body electrical conductivity (tobec) to estimate total body fat of free-living birds. *Condor* **92**:469-499.

Clayton,N.S. and Birkhead,T.R. 1989. Consistency in the scientific name of the zebra finch. *Auk* 106:750.

Davies, S.J.J.F. 1977. The timing of breeding by the zebra finch *Taeniopygia castanotis* at Mileura, Western Australia. *Ibis* **119**: 369-372.

Donnan,D 1994. The nutritional requirements of egg production in zebra finches. PhD thesis, Glasgow University.

Drent, R.H. and Daan, S. 1980. The prudent parent: energetic adjustments in avian breeding. *Ardea* **68**: 225-52.

El-Wailly, A.J. 1966. Energy requirements for egg-laying and incubation in the zebra finch *Taeniopygia castanotis. Condor* **68**: 582-594.

Ettinger, A.O. and King, J.R. 1979. Time and energy budgets of the willow flycatcher *Empidonax trailli* during the breeding season. *Auk* **97**: 535-546.

Fogden,M.P.L., and Fogden,P.M. 1979. The role of fat and protein reserves in the annual cycle of the grey-backed cameroptera in Uganda. *J.Zool.,Lond.* **189**:233-58.

Freeman,S. and Jackson,W.M. 1990. Univariate metrics are not adequate to measure avian body size. *Auk* **107**:69-74.

Hails,C.J. and Turner,A.K. 1985. The role of fat and protein during breeding in the white-bellied swiftlet (*Collocalia esculenta*). *J.Zool.,Lond.***206**:469-484.

Harvey, D 1970. Tables of the amino acids in foods and feeding stuffs. Technical Communication No 19. Edinburgh: Commonwealth Bureau of Animal Nutrition.

Haywood, S. 1993a. Sensory control of clutch size in the zebra finch *Taeniopygia guttata. The Auk* **110**: 778-786.

Haywood, S. 1993b. Sensory and hormonal control of clutch size in birds. *Q. Rev. Biol.* **68**: 33-60.

Haywood, S. 1993c. Role of extrinsic factors in the control of clutch-size in the blue tit *Parus caeruleus. Ibis* **135**: 79-84.

Haywood, S. and Perrins, C.M. 1992. Is clutch size in birds affected by environmental conditions during growth? *Proc. R. Soc. Lond. B.* **249**: 195-197.

Hohman,W.L. and Taylor,T.S. 1986. Indices of fat and protein for ring-necked ducks. *J.Wildl.Mgmt.***50**:209-211.

Hornfeldt, B. and Eklund, U. 1990. The effect of food on laying date and clutch-size in Tengmalm's owl (*Aegolius funereus*). *Ibis* **132**: 395-406.

Houston,D.C., Donnan,D. and Jones,P.J. in press *a*. The source of the nutrients required for egg production in zebra finches *Poephila guttata*. *J.Zool.,Lond.*.

Houston,D.C., Donnan,D. and Jones,P.J. in press b. Use of labelled methionine to investigate the contribution of muscle proteins to egg production in zebra finches. *J.Comp.Physiol.*B

Houston,D.C., Donnan,D., Jones,P., Hamilton,I. and Osborne,D. in press. Changes in the muscle condition of female zebra finches *Poephila guttata* during egg laying and the role of protein storage in bird skeletal muscle. *Ibis*.

Houston,D.C., Jones,P.J. and Sibly,R.M. 1983. The effect of female body condition on egg laying in lesser black-backed gulls *Larus fuscus*. *J. Zool.,Lond.***200**:509-520.

Hoyt, D.F. 1979. Practical methods of estimating volume and fresh weight of bird eggs. *Auk* **96**: 73-77.

Jarvinen, A. and Ylimaunu, J. 1984. Significance of egg-size on the growth of nestling pied flycatchers *Ficedula hypoleuca*. *Annals Zoologica Fennica* **21**: 213-216.

Johnstone, R.D. 1993. The effect of direct supplementary feeding of nestlings on weight loss in female great tits *Parus major*. *Ibis* **135**: 311-314.

Jones,M.M. 1980. Nocturnal loss of muscle protein from house sparrows (*Passer domesticus*). *J.Zool., Lond.* **192**:33-39.

Jones,M.M. 1991. Muscle protein loss in laying house sparrows, *Passer domesticus*. *Ibis* **133**:193-8.

Jones,P.J. and Ward,P. 1976. The level of reserve protein as the proximate factor controlling the timing of breeding and clutch size in the red-billed quelea. *Ibis* **118**:547-74.

Jones, P.J and Ward, P 1979. A physiological basis for colony desertion by red-billed queleas (*Quelea quelea*). *J. Zool., Lond.* **189**: 1-19.

Kendall,M.D., Ward,P. and Bacchus,S. 1973. A protein reserve in the pectoralis major flight muscle of *Quelea quelea. Ibis* **115**:264-70.

Korschgen,C.E. 1977. Breeding stress of female eiders in Maine. *J.Wildl.Mgmt.* 41:360-373.

Magrath, R.D. 1992. The effect of egg mass on the growth and survival of blackbirds: a field experiment. *Journal of Zoology, London* **227**: 639-653.

Millward, D.J., Garlick, P.J., Nnanyelugo, D.O. and Waterlow, J.C. 1976. The relative importance of muscle protein synthesis and breakdown in the regulation of muscle mass. *Biochem. J.* **156**: 185-8.

Morton,S.R. and Davies,P.H. 1983. Food of the zebra finch (*Poephila guttata*) and an examination of granivory in birds of the Australian arid zone. *Aust.J.Ecol.***8**:235-243.

Murphy,M.E. and King, J.R. 1990. Diurnal changes in tissue glutathione and protein pools of moulting white-crowned sparrows: the influence of photoperiod and feeding schedule. *Physiol.Zool.***63**:1118-1140.

Nilsson,J-A. and Svenson,E. 1993. Energy constraints and ultimate decisions during egg laying in the blue tit. *Ecology* **74**: 244-251.

Nisbet, I.C.T 1978. Dependence of fledging success on egg-size, parental performance and egg-composition among common and roseate terns *Sterna hirundo* and *S. dougallii. Ibis* **120**: 207-215.

O'Connor, R.J. 1979. Egg weights and brood reduction in the European swift *Apus apus. Condor* **81**: 133-145.

Ojanen, M. 1983. Composition of the eggs of the great tit *Parus major* and the pied flycatcher *Ficidula hypoleuca. Annals Zoologica Fennica* **20**: 57-63.

Perrins, C.M. 1965. Population fluctuation and clutch-size in the great tit *Parus major. J. Anim. Ecol.* **34**: 604-647.

Perrins, C.M. and McCleery, R.H. 1989. Laying dates and clutch-size in the great tit. *Wilson Bull.* **101**: 236-253.

Perrins, C.M. and McCleery, R.H. 1994. Competition and egg-weight in the great tit *Parus major*. *Ibis* **136**: 454-456.

Pethig,R. 1979. Dielectric and electronic properties of biological materials. Wiley, Chichester.

Reid, W.V. and Boersma, P.D. 1990. Parental quality and selection on egg-size in the magellanic penguin. *Evolution* **44**: 1780-1786.

Rising,J.D. and Somers,K.M. 1989. The measurement of overall body size in birds. *Auk* **106**:666-674.

Rofstad, G. and Sandvik, J. 1987. Morphology of hatchling hooded crows and its relation to egg volume. *Condor* **89**: 494-499.

Sawicka-Kapusta,K. 1975. Fat extraction in the Soxhlet apparatus. In *Methods for ecological bioenergetics*:288-292. I.B.P. handbook no. 24.

Schifferli, L. 1973. The effect of egg weight on the subsequent growth of nestling great tits *Parus major*. *Ibis* **115**: 549-558.

Scott, M.L., Neshein, M.C. and Young, R.J. 1982. Nutrition of the chicken. M.L. Scott & Associates, New York.

Sears,J. 1988. Assessment of body condition in live birds; measurements of protein and fat reserves in the mute swan, *Cygnus olor*. *J.Zool.,Lond.***216**:295-308.

Sibly,R.M., Jones,P.J. and Houston,D.C. 1987. The use of body dimensions of lesser black-backed gulls *Larus fuscus* to indicate size and to estimate body reserves. *Functional Ecology* **1**:275-279.

Skagen, S.K. 1988. Asynchronous hatching and food limitation: a test of Lack's hypothesis. *The Auk* **105**: 78-88.

Taborsky, M. 1994. The functional significance of shared incubation. *J. fur Ornith.* **135**: 273.

Walsberg,G.E. 1988. Evaluation of a nondestructive method for determining fat stores in small birds and mammals. *Physiol.Zool.***61**:153-159.

Ward,P. 1969. The annual cycle of the yellow-vented bulbul *Pycnotus goiavier* in a humid equatorial environment. *J.Zool.,Lond.***157**:25-45.

Williams, T.D. 1994. Intrapecific variation in egg size and egg composition in birds: effects on offspring fitness. *Biol. Rev.* **68**: 35-59.

Zann,R. and Straw,B. 1984. Feeding ecology and breeding of zebra finches in farmland in northern Victoria. *Aust.Wildl.Res.***11**:533-52.

Appendix 1

A comparison of (a) clutch sizes, and (b) mean egg masses and clutch weights between females that were on a diet of "high quality protein" (HQP) and those on "low quality protein" (LQP) prior to breeding.

(a)

	HQP			LQP			
Variable	Median	IQR	n	Median	IQR	n	Significance of difference
Clutch size	5	2	27	3	3	26	U=175, Z=-3.19 P=0.001

(b)

	HQP			LQP			
Variable	Mean	SE	n	Mean	SE	n	Significance of difference
Mean egg mass (g)	1.062	0.016	26	0.936	0.030	23	t_{47}=3.83 P<0.001
Clutch mass (g)	4.825	0.393	26	2.601	0.305	26	t_{50}=4.47 P<0.001

Appendix 2

a) **Clutch size** - correlations with the size of body reserves, and the change in reserves, for females that received either a diet of "high quality protein" (HQP) or "low quality protein" (LQP) prior to breeding. Body reserves were measured before and after laying.

Variable	HQP	LQP
Pre-lay body mass (g)	r_{27}=-0.257, P=0.195	r_{26}=0.014, P=0.945
Pre-lay body condition	r_{27}=-0.216, P=0.279	r_{26}=-0.102, P=0.620
Pre-lay muscle mass (g)	r_{27}=-0.001, P=0.996	r_{26}=0.162, P=0.428
Pre-lay muscle condition	r_{27}=0.087, P=0.665	r_{26}=0.014, P=0.947
Post-lay body mass (g)	r_{25}=-0.390, P=0.054	**r_{20}=-0.503, P=0.024**
Post-lay body condition	r_{25}=-0.359, P=0.078	**r_{20}=-0.451, P=0.046**
Post-lay muscle mass (g)	r_{25}=-0.293, P=0.156	r_{20}=-0.197, P=0.406
Post-lay muscle condition	r_{25}=-0.261, P=0.207	r_{20}=-0.147, P=0.536
Change in muscle mass(g)	**r_{25}=-0.485, P=0.014**	r_{20}=-0.083, P=0.714
Change in body mass(g)	r_{25}=-0.178, P=0.395	r_{20}=-0.352, P=0.128

Appendix 2

b) **Mean egg mass** - correlations with the size of body reserves, and the change in reserves, for females that received either a diet of "high quality protein" (HQP) or "low quality protein" (LQP) prior to breeding. Body reserves were measured before and after laying.

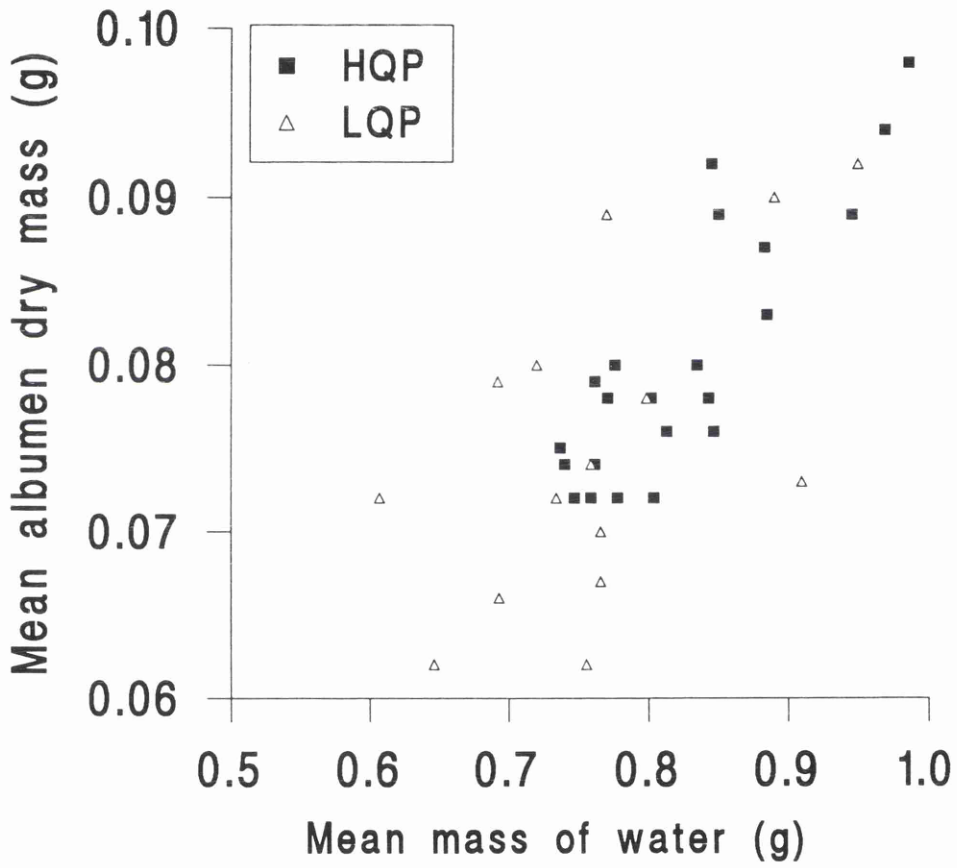
Variable	HQP	LQP
Pre-lay body mass (g)	r_{26}=-0.146, P=0.478	r_{23}=-0.165, P=0.451
Pre-lay body condition	r_{26}=-0.215, P=0.292	r_{23}=-0.274, P=0.206
Pre-lay muscle mass (g)	r_{26}=0.157, P=0.444	r_{23}=-0.051, P=0.816
Pre-lay muscle condition	r_{26}=0.097, P=0.638	r_{23}=-0.162, P=0.461
Post-lay body mass (g)	r_{25}=0.095, P=0.652	r_{20}=0.266, P=0.258
Post-lay body condition	r_{25}=0.054, P=0.799	r_{20}=0.266, P=0.256
Post-lay muscle mass (g)	r_{25}=0.151, P=0.472	r_{20}=0.059, P=0.804
Post-lay muscle condition	r_{25}=0.081, P=0.701	r_{20}=0.055, P=0.819
Change in muscle mass(g)	r_{25}=-0.051, P=0.809	r_{20}=0.009, P=0.970
Change in body mass(g)	r_{25}=0.263, P=0.204	**r_{20}=0.555, P=0.011**

Appendix 2

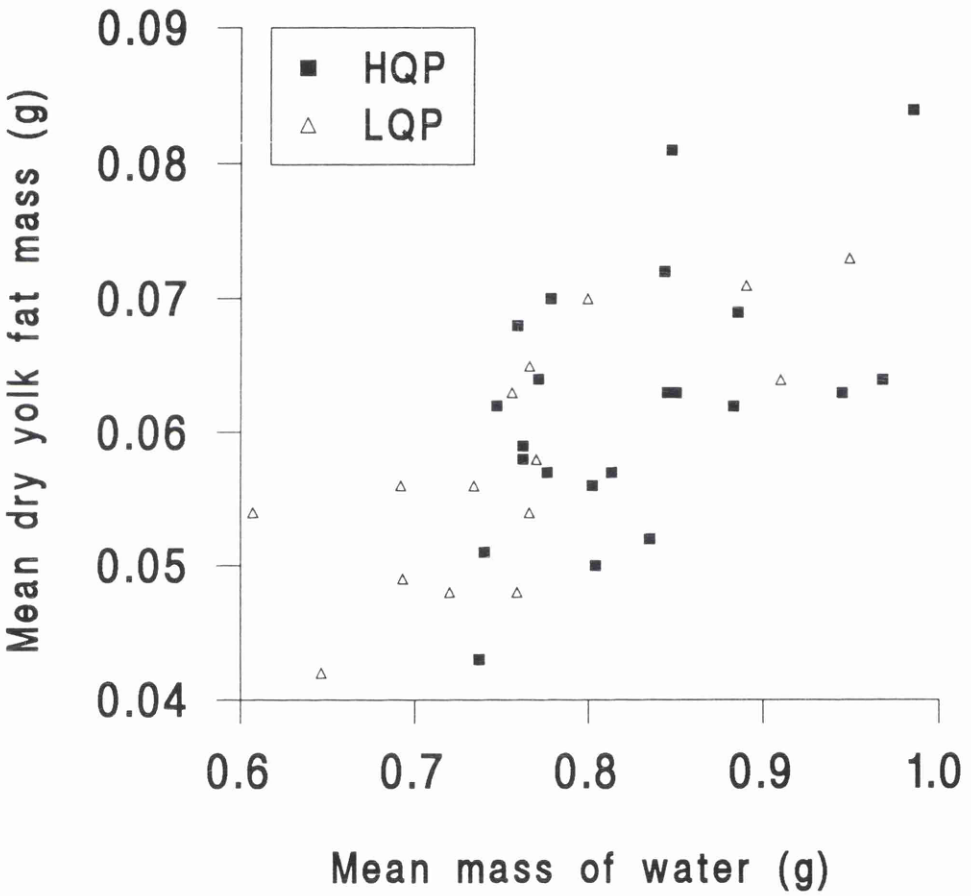
c) **Clutch mass** - correlations with the size of body reserves, and the change in reserves, for females that received either a diet of "high quality protein" (HQP) or "low quality protein" (LQP) prior to breeding. Body reserves were measured before and after laying.

Variable	HQP	LQP
Pre-lay body mass (g)	r_{26}=-0.332, P=0.097	r_{26}=-0.066, P=0.749
Pre-lay body condition	r_{26}=-0.309, P=0.125	r_{26}=-0.145, P=0.480
Pre-lay muscle mass (g)	r_{26}=-0.060, P=0.771	r_{26}=0.205, P=0.316
Pre-lay muscle condition	r_{26}=-0.018, P=0.929	r_{26}=0.119, P=0.564
Post-lay body mass (g)	r_{25}=-0.345, P=0.091	r_{20}=-0.425, P=0.062
Post-lay body condition	r_{25}=-0.338, P=0.098	r_{20}=-0.389, P=0.090
Post-lay muscle mass (g)	r_{25}=-0.316, P=0.124	r_{20}=-0.230, P=0.329
Post-lay muscle condition	r_{25}=-0.302, P=0.142	r_{20}=-0.190, P=0.423
Change in muscle mass(g)	**r_{25}=-0.400, P=0.047**	r_{20}=-0.135, P=0.571
Change in body mass(g)	r_{25}=-0.111, P=0.599	r_{20}=-0.123, P=0.605

Appendix 3a. Comparison of the albumen dry masses of eggs from HQP and LQP females. Mean clutch data were used. Gradients $F_{1,32}=0.36$, $P=0.550$; elevations $F_{1,33}=0.09$, $P=0.761$.



Appendix 3b. Comparison of the mean dry yolk masses of eggs from HQP and LQP females. Mean clutch data were used. Gradients $F_{1,32}=0.00$, $P=0.966$; elevations $F_{1,33}=0.06$, $P=0.808$.



Appendix 3c. Comparison of the mean lean dry yolk masses of HQP and LQP females. Clutch means were used. Gradients $F_{1,32}=0.77$, $P=0.386$; elevations $F_{1,33}=0.15$, $P=0.700$.